

***HELICOBACTER PYLORI* IN VAMMALA, FINLAND:
SEROEPIDEMIOLOGICAL STUDIES AND A POPULATION-
BASED ‘SCREEN-AND-TREAT’ PROGRAMME**

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Academic Dissertation

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in the Small Auditorium of Haartman Institute, Haartmaninkatu 3, Helsinki,
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals, and reprinted with the permission of the copyright holders, and on unpublished data (I, II).

- I. Salomaa-Räsänen A, Kosunen TU, Aromaa A, Knekt P, Sarna S, Rautelin H. A 'screen-and-treat' approach for *Helicobacter pylori* infection – a population-based study in Vammala, Finland. Submitted.
- II. Salomaa-Räsänen A, Kosunen TU, Mattila J, Sarna S, Rautelin H. Age-dependent accuracy of *Helicobacter pylori* antibody assays in adults with special emphasis on atrophic gastritis. Clin Diagn Lab Immunol 2004; 11: 1185-1188.
- III. Salomaa-Räsänen A, Kosunen T, Karjalainen J, Aromaa A, Knekt P, Sarna S, Rautelin H. IgA antibodies in persisting *Helicobacter pylori* infection in Finnish adults. Clin Microbiol Infect 2006; 12: 236-240.
- IV. Perez-Perez GI, Salomaa A, Kosunen TU, Daverman B, Rautelin H, Aromaa A, Knekt P, Blaser MJ. Evidence that *cagA*⁺ *Helicobacter pylori* strains are disappearing more rapidly than *cagA*⁻ strains. Gut 2002; 50: 295-298.

ABBREVIATIONS

BabA	Blood group antigen binding adhesion A
b.i.d.	bis in die (twice a day)
<i>cagA</i>	cytotoxin-associated gene
CagA	protein coded by <i>cagA</i>
CI	confidence interval
DNA	deoxyribonucleic acid
DOB	delta over baseline
<i>dupA</i>	duodenal ulcer promoting gene A
EIA	enzyme immunoassay
GP	primary care physician
GORD	gastro-oesophageal reflux disease
<i>H. pylori</i>	<i>Helicobacter pylori</i>
IgA, E, G, M	immunoglobulin A, E, G, M
IL-8, -10, -1 β	interleukin 8, 10, 1 β
MALT	mucosa-associated lymphoid tissue
NSAID	nonsteroidal anti-inflammatory drug
ODR	optical density ratio
OipA	outer inflammatory protein A
OR	odds ratio
PAI	pathogenity island
PCA	parietal cell antibody
PCR	polymerase chain reaction
PG I, II	pepsinogen I and II
PPI	proton pump inhibitor
q.i.d.	quarter in die (four times a day)
RCT	ranomized controlled trial
RUT	rapid urease test
t.i.d.	ter in die (three times a day)
UBT	urea breath test
<i>vacA</i>	gene coding for vacuolating toxin
VacA	vacuolating toxin

ABSTRACT

Background: The age-adjusted *Helicobacter pylori* (*H. pylori*) seroprevalence rate in the adult population decreased from 51% to 34% from 1973 to 1994 in Vammala, Finland, before the current use of antimicrobials in the treatment of helicobacter gastritis. Almost all *H. pylori* infected individuals show elevated levels of specific immunoglobulin G (IgG) antibodies, but in about two-thirds of subjects the specific IgA antibodies were elevated. Presence of *H. pylori* IgA antibodies has been associated with a CagA-positive infection, which in turn is associated with an increased risk of severe complications of *H. pylori* infection.

Aims: To accelerate the decline of *H. pylori* infection, and to examine risk factors for *H. pylori* infection in Finnish young adults, we started a unique population-based, voluntary 'screen-and-treat' programme in primary health care practice, first as a pilot study in 1994 and since 1996 extending the programme to include over 6000 young adults in Vammala, Finland. We aimed to determine the accuracy of *H. pylori* IgG and IgA antibody tests for adults in different age-groups, with special emphasis on the presence of atrophic gastritis. The same enzyme immunoassay (EIA) tests were used in the 'screen-and-treat' programme. We also studied whether an increase in the number of IgA antibody-positive *H. pylori* patients with respect to age is a cohort phenomenon only, and how the elevated IgA antibody levels changed during infection, and determined whether the proportion of subjects infected with CagA⁺ or CagA⁻ *H. pylori* strains has changed as the overall prevalence of *H. pylori* has declined.

Participants and methods: A total of 3326 individuals aged 15-40 in 1996, and 716 subjects aged 15 and 584 aged 45 in 1997-2000 were screened for *H. pylori* infection using serology. Altogether 4626 (75.7%) inhabitants participated in screening. Serum IgG and IgA antibodies to *H. pylori* were determined and all antibody-positive subjects were offered eradication therapy. The first-line treatment included: amoxicillin 1g b. i. d., metronidazole 400mg t. i. d., and lansoprazole 30mg b. i. d. for seven days. Retreatment for those whose first treatment failed, included amoxicillin 1g, clarithromycin 500mg and lansoprazole 30mg b. i. d. for seven days.

The cure was verified by serology and occasionally by the ^{13}C -Urea breath test (UBT). A total of 561 consecutive adult patients were gastroscopied for clinical indications. Biopsy samples were taken for histology and culture, and serum samples were tested for *H. pylori* IgG and IgA antibodies and pepsinogen I (PGI) levels. Results were considered helicobacter positive if culture or histology were positive. Paired serum samples collected from 224 subjects in both 1973 and 1994 and paired serum samples from 336 persons collected in both 1978-1980 and 1997-1998, stored at -20°C , were reanalysed for *H. pylori* IgA antibodies using in-house assays for the 224 subjects and Pyloriset EIAs for the 336 persons. Serum samples collected from 408 subjects in 1973, from 503 subjects in 1994, and paired serum samples from 221 subjects collected both in 1973 and 1994, stored at -20°C , were reanalysed for CagA antibodies using a recombinant CagA fragment by EIA.

Results: *H. pylori* seroprevalence rates were 39% in the pilot study, 12% among subjects aged 15-40 years in 1996, and 3% and 27% among the 15 and 45-year-old subjects in 1997-2000, respectively. In the 'screen-and-treat' programme, the eradication rates were 89.8% in the pilot study, 82.2% and 77.6% per-protocol and 71.9% and 62.9% in intention-to-treat in subjects invited in 1996 and 1997-2000, respectively. *H. pylori* seroprevalence rates were calculated to have decreased to 19% in the pilot study, to 4% among subjects aged 15-40 years, and to 2% and 12% among the 15 and 45-year-old subjects, respectively. In multivariate analysis, crowding variables in the childhood household, low education of the mother, current smoking and alcohol consumption, unfavourable housing conditions, and sick leave due to dyspepsia were independent risk factors for *H. pylori* infection. The *H. pylori* antibody test for IgG was highly accurate in subjects aged 15-49, but the specificity declined in older age-groups, in which the prevalence of atrophic gastritis increased. The sensitivity of the IgA test increased and the specificity decreased by age. The exclusion of 'false' positive results for patients with atrophic gastritis clearly improved the specificities of both the tests for older age-groups. The number of IgA-positive subjects increased during the follow-up and there was an increase in elevated IgA antibody levels, as well. The proportion of subjects infected with CagA⁺ *H. pylori* strains had declined more rapidly than the proportion of subjects infected with CagA⁻ *H. pylori* strains in subjects aged 14-44.

Conclusions: The primary health care system of Finland provided good possibilities to organize this large-scale programme. It remains to be seen, how much this intervention will reduce the burden of *H. pylori* associated diseases and related health costs in this low prevalence area. Serologic assays based on IgG antibodies were highly accurate in young age-groups and also among the elderly when those with atrophic gastritis were excluded. The increase in the number of IgA antibody-positive *H. pylori* patients with age was due not only to the birth cohort phenomenon and seroconverters, but also to increasing IgA antibody levels during infection. It remains to be seen in the future what are the clinical consequences of the rapid decline in CagA⁺ *H. pylori* infection in subjects aged 14-44 years.

INTRODUCTION

Helicobacter pylori infection is a chronic infection which is mainly acquired in childhood (Kosunen et al., 1997). The prevalence rates of *H. pylori* antibodies increase with age in all populations (Mégraud, 1993). This has been explained by a so-called cohort phenomenon: the persistence of childhood infections, which have gradually decreased since the early 1900s (Banatvala et al., 1993a; Sipponen et al., 1996). Low socioeconomic status, crowded living conditions and a lack of hot tap water are risk factors for acquisition of the infection in early childhood (Malaty, 2007). *H. pylori* infection is chronic and does not usually heal without specific eradication treatment. Almost all *H. pylori* infected subjects have elevated levels of specific IgG antibodies, but only in about two thirds of them does the IgA titer exceed the cut-off level (Kosunen et al., 1992). An IgA antibody response during *H. pylori* infection has been associated with peptic ulcer disease and gastric cancer (Aromaa et al., 1996; Kosunen et al., 2005). In addition, the production of IgA antibodies is found to be associated with a CagA positive infection (Rautelin et al., 2000) which in turn is associated with an increased risk of severe complications of the infection, like peptic ulcer disease, atrophic gastritis and intestinal metaplasia, and gastric cancer (Cover et al., 1995; Kuipers et al., 1995; Parsonnet et al., 1997).

Recommendations for the diagnostic methods used for detecting *H. pylori* infection have changed and now it is even generally accepted that the role of serology in detecting the infection is crucial. The latest European guidelines on the management of *H. pylori* infection, the Maastricht III Consensus Report, emphasized the special role of serology compared to other diagnostic tests (Malfertheiner et al., 2007). Proton pump inhibitor (PPI) treatment prior to testing can lead to false negative results in other diagnostic tests than serological assays. Serological tests are recommended to diagnose *H. pylori* infection also in patients suffering from bleeding ulcers and conditions with a low bacterial density (gastric atrophy, mucosa-associated lymphoid tissue (MALT) lymphoma); both invasive and other non-invasive tests have demonstrated a limited sensitivity in those patients. Quantitative serological assays are reliable to be used also in the follow-up after eradication treatment. The limitation of this method is that it may demonstrate a lower performance if the population has

been actively treated for *H. pylori* infection, since after successful *H. pylori* eradication therapy the antibodies decline slowly to normal levels (Veijola et al., 2007).

The indications for eradication treatment have widened. According to the latest European guidelines, strongly recommended indications for eradication therapy are peptic ulcer disease, bleeding peptic ulcer, low-grade gastric MALT lymphoma, atrophic gastritis, post-gastric cancer resection, gastric cancer in close relatives, and patient's wishes. Indications for treatment are also long-term maintenance treatment with PPIs, start of using nonsteroidal anti-inflammatory drugs (NSAIDs), unexplained iron deficiency anaemia and idiopathic thrombocytopenic purpura, according to the latest guidelines. *H. pylori* 'test and treat' was regarded appropriate for patients with uninvestigated dyspepsia (Malfertheiner et al., 2007).

Although it has been suggested that *H. pylori* eradication for gastric cancer prevention in populations at risk should be evaluated (Malfertheiner et al., 2007), screening and treating the general population for *H. pylori* has not yet been recommended. Although the prevalence of *H. pylori* infection is steadily declining in developed countries, also in Finland (Rehnberg-Laiho et al., 2001; Malaty, 2007), and the prevalence of *H. pylori* associated peptic ulcer disease has declined (Veijola et al., 2005a; Koivisto et al., 2005), the severe complications of the infection still exist. If a screening and treating for *H. pylori* programme could be offered to young adults in the general population, it would be possible to cure the infection before the development of pre-neoplastic conditions and also the transmission of the infection from parents to children could be prevented.

REVIEW OF THE LITERATURE

HISTORY OF *H. PYLORI*

The bacterium *H. pylori* has been present for at least 3000 years. The presence of gastric spiral bacteria was first documented in 1893 by an Italian pathologist Bizzozero, who found these bacteria in the stomach of dogs (Bucley et al., 1998). In the human stomach the bacteria were demonstrated in 1906 (Bucley et al., 1998). Pathologist Robin Warren noticed helicobacters in 1979 during routine diagnostic procedures, and in 1981 after having met Barry Marshall the modern story of *H. pylori* and peptic ulcer disease began (Marshall and Warren, 1983; Bucley et al. 1998). Warren and Marshall found the association between Campylobacter like organisms and gastritis and peptic ulceration and succeeded even to cultivate this slow-growing organism. They also started the modern eradication of *H. pylori* with antimicrobial agents, and were the first to suggest the possibility of the association of *H. pylori* with gastric cancer (Marshall and Warren, 1983; Marshall and Warren, 1984). A causative association between *H. pylori* and gastritis was proven when Marshall and Morris independently ingested the bacterium and observed the results fulfilling the prerequisites of Koch's postulates (Marshall et al., 1985; Morris and Nicholson, 1987). Warren and Marshall were awarded with the Nobel Prize in Medicine in 2005 emphasizing the importance of their observations on *H. pylori* and peptic ulcer disease.

BACTERIOLOGY OF *H. PYLORI*

1. Bacteriology

H. pylori is a gram-negative, spiral-shaped, multi-flagellated bacterium that infects the human gastric mucosa. It is microaerophilic and relatively slowly growing in

culture, where it usually occurs in the form of straight or slightly curved rods (Goodwin et al., 1985). Coccoidal forms of the bacterium may be seen after prolonged culture (Mégraud et al., 1985). *H. pylori* is identified in culture by colony appearance, Gram staining, and positive reactions for oxidase, urease, and catalase.

H. pylori has unique ability to survive in the acid environment of stomach due to the bacterium's ability to produce urease enzyme which splits urea to ammonium, and due to an acid-regulated urea transport channel (Weeks et al., 2000). The bacterium can also escape the gastric acidity with the aid of flagellae and penetrate into the gastric mucus (Owen, 1998).

The genus *Helicobacter* comprises of 31 formally named different species (List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.cict.cict.fr> accessed 26th April 2008).

2. CagA and other virulence factors

Initial *H. pylori* colonization occurring mostly in childhood proceeds to chronic persistent infection and inflammation, and in some of the infected individuals to peptic ulcer disease, premalignant conditions and rarely to gastric cancer. The progression of the infection depends on strain virulence, host genetic susceptibility and environmental factors.

H. pylori strains are either CagA-positive or CagA-negative based on whether they produce a highly immunogenic 128 kDa protein, which is encoded by the *cagA* gene. Cytotoxin-associated gene *cagA* is a marker of the pathogenicity island (PAI), which encodes disease-associated virulence factors of *H. pylori* (Censini et al., 1996; Akopyants et al., 1998). CagA is present in 40 to 70% of *H. pylori* strains, and in the Western world it is associated with peptic ulcers, precancerous conditions like atrophic gastritis and gastric cancer according to several studies (Cover et al., 1995; Kuipers et al., 1995; Parsonnet et al., 1997; Kusters et al., 2006). CagA-positive strains have also been reported to associate with non-ulcer dyspepsia (Ching et al., 1996; Loffeld et al., 2001). *H. pylori* strains possessing the *cagPAI* activate epithelial

cell lines to express pro-inflammatory cytokine interleukin-8 (IL-8) and *cag*⁺-strains are associated with high levels of epithelial cell IL-8 expression (Crabtree et al., 1995; Peek et al., 1995). A serological response to the CagA-protein (an elevated level of CagA antibodies) is strongly predictive that a person is carrying a CagA-positive *H. pylori* strain (Blaser et al., 1995).

Cytotoxin VacA encoded by the gene *vacA* is another well-known virulence factor (Leunk, 1991). It is associated with peptic ulcer and adenocarcinoma. The gene *vacA* is present in all strains, but due to several alleles (Atherton et al., 1995) various amounts of toxins are produced and about half of *H. pylori* strains secrete the toxin VacA. Genotype s1m1 is the most virulent genotype of *vacA* and it is associated with the *cagA* positive genotype (Kusters et al., 2006). There are also other putative virulence factors. The expression of *H. pylori* outer inflammatory protein A (OipA) is associated with duodenal ulceration and gastric cancer (Robinson et al., 2007). Duodenal ulcer promoting gene A (*dupA*) is associated with duodenal ulceration in patients from South Korea, Japan, and Colombia, but seemed to protect against gastric cancer in these populations (Robinson et al., 2007). Expression of Blood group antigen binding adhesion A (BabA) increases the association of *cag*⁺ strains with duodenal ulceration and gastric cancer in some people (Robinson et al., 2007).

HOST RESPONSE TO *H. PYLORI*

H. pylori colonisation in human stomach usually occurs in childhood, but in most subjects the infection persists lifelong without an effective treatment. All infected individuals develop an inflammatory and immune response to the bacterium, but the response is not able to cure the infection. According to self inoculation studies in adults, acute *H. pylori* infection is usually symptomatic, creating epigastric discomfort, nausea, malaise, belching and halitosis, and the symptoms usually resolve within two weeks (Marshall et al., 1985; Morris and Nicholson, 1987; Sobala et al., 1991). Histologically, an acute infection produces a heavy neutrophilic infiltration and a gradual infiltration of inflammatory cells of all classes, predominantly lymphocytes,

which persists, and a transient hypochlorhydria (Marshall et al., 1985; Morris and Nicholson, 1987; Sobala et al., 1991). Since spontaneous cure of the infection is rare (Valle et al., 1996; Kosunen et al., 1997) the infection proceeds to chronic gastritis in most individuals, characterized predominantly by lymphocytes.

The infection induces an immunological response with circulating antibodies, which is unable to eradicate the bacterium from the gastric mucosa. Most helicobacter-infected subjects have specific circulating IgG antibodies, but systemic IgA antibodies for *H. pylori* are less common and are elevated in approximately two-thirds of infected subjects (Kosunen et al., 1992). Of infected individuals, 2-7% show an elevated IgA level alone (Kosunen et al., 1992; Kosunen et al., 1997; Jaskowski et al., 1997). The prevalence rates of IgG and IgA antibodies increase by age (Kosunen et al., 1989; Andersen et al., 1996). Occurrence of specific IgM antibodies is especially associated with new infections with *H. pylori* (Andersen et al., 1996). The clinical importance of the IgA antibody response during *H. pylori* infection is emphasized when considered in association with earlier findings showing an association between *H. pylori* IgA and peptic ulcer disease, gastric cancer (Aromaa et al., 1996; Kosunen et al., 2005) and a CagA-positive infection (Rautelin et al., 2000).

The immune response to *H. pylori* includes release of pro-inflammatory and antibacterial factors and infiltration of all classes of immune effector cells in the gastric mucosa. The pro-inflammatory cytokine IL-8 recruits lymphocytes and neutrophils, and interleukin 1 β (IL-1 β) is responsible for activation of macrophages and polymorphonuclear leukocytes (Robinson et al., 2007). In addition, IL-1 β is a potent inhibitor of acid secretion (Kusters et al., 2006). There is a life-long persisting local and systemic acquired humoral and cell-mediated response to *H. pylori*. IL-10-producing T-cells are regarded to be essential in the control of the inflammation (Kusters et al., 2006). The T-cell response is mainly of Th 1 type. It is suggested using animal models that Th 1 differentiation is important in pathogenesis of the infection (Robinson et al., 2007).

EPIDEMIOLOGY OF *H. PYLORI* INFECTION

1. Geographical distribution and the prevalence of *H. pylori* infection.

The cohort phenomenon.

Infection with *H. pylori* is worldwide. The prevalence of *H. pylori* varies between countries with large differences between developed and developing countries (Mégraud, 1993; Talley et al., 1993). In developing countries the prevalence of *H. pylori* infection is much higher than in developed countries, and the epidemiology of the infection is characterised by a rapid acquisition rate of the infection so that even 80% of the population acquires the infection by the age of 20 (Graham et al., 1991a). Low socioeconomic status and close person-to-person contact increase the likelihood of contracting this infection and also in developed countries most of the infected people have got the infection in childhood (Webb et al., 1994). The prevalence rates of the infection increase with age in all populations (Mégraud, 1993). This has been explained by the persistence of childhood *H. pylori* infections, which have decreased gradually since the early 1900s: the so-called birth-cohort phenomenon (Parsonnet et al., 1992; Banatvala et al., 1993a; Sipponen et al., 1996; Kosunen et al., 1997). In developed countries the acquisition rate of new infections in adults is estimated to be less than 3% per 10 years (Mégraud, 1993; Kosunen et al., 1997). The prevalence of the infection varies between subpopulations in the same country (Graham et al., 1991b; Breuer et al., 1996; Everhart et al., 2000). In Finland the age-adjusted seroprevalence rate in adult population was 34% in 1994 (Kosunen et al., 1997). The seroprevalence of the infection was 5.6% in Finnish children aged 2 to 20 years, among whom the majority of new infections were acquired before the age of seven years (Rehnberg-Laiho et al., 1998), while the prevalence was 66% in Finnish centenarians (Rehnberg-Laiho et al., 1999).

2. Changing prevalence of *H. pylori* infection

The prevalence of *H. pylori* infection is steadily declining in developed countries (Rehnberg-Laiho et al., 2001; Malaty, 2007). In countries, where living standards have rapidly improved, the prevalence of *H. pylori* infection in children has

dramatically decreased. In Estonia, the age standardized seroprevalence rate in children decreased from 42.2% in 1991 to 28.1% in 2002 ($p = 0.0002$) during the 11-year period of profound socioeconomic changes in Estonia. Stratified analysis by the age-groups demonstrated that a statistically significant decrease in seroprevalence occurred in children aged 0.5-5 years ($p < 0.005$) and in children aged 6 to 10 years ($p < 0.05$) (Oona et al., 2004). In Russia results from two cross-sectional studies among children in St. Petersburg demonstrated, how the prevalence among children younger than 5 years of age decreased from 30% in 1995 to 2% a decade later in the same age-group ($p = 0.001$) (Malaty, 2007), indicating that improvements in the standard of living had resulted in a marked reduction in *H. pylori* transmission, according to the author.

3. Social class and living conditions as risk factors for *H. pylori* infection

Low socioeconomic status and crowded living conditions are risk factors for acquisition of the infection in early childhood (Malaty and Graham, 1994; Everhart et al., 2000; Koch et al., 2005; Bures et al., 2006). The prevalence of *H. pylori* infection is inversely related to socioeconomic status in childhood (Graham et al, 1991b) irrespective of the present social class (Malaty and Graham, 1994). Occupation, family income level and living conditions in childhood home are parts of a same socioeconomic complex explaining the risks of becoming infected. For populations in which the social class is homogenous, the density of living conditions is the most significant risk factor (Malati et al., 1996).

4. Transmission of *H. pylori*

Transmission of *H. pylori* infection has been addressed through prevalence studies within families, detection in fecal and oral environments, detection in environments, and direct inference from strain similarity (Delpont and Merwe, 2007). Especially in developing countries, the low quality of drinking water or a lack of hot tap water have been suggested to contribute to transmission of the infection. *H. pylori* has been identified in drinking water (Schauer et al., 1995). In addition, municipal water supply

has been suggested for an infection route (Klein et al., 1991) and a transmission via uncooked vegetables treated with sewage has been proposed (Hopkins et al., 1993). Transmission via inadequately disinfected endoscopy equipment has been demonstrated (Stone, 1999). However, it appears likely that *H. pylori* is transmitted from person-to-person. Comparison of DNA fingerprints provides evidence for intrafamilial transmission of the infection (Bamford et al., 1993). Especially an infected mother is found to be a risk factor for acquiring the infection in childhood both in a developing country and in a developed country (Aguemon et al., 2005; Kivi et al., 2005). In Sweden, infected siblings were found to be a risk factor for acquiring the infection (Kivi et al., 2005). The possible person-to-person transmission routes are gastro-oral, oral-oral and fecal-oral routes, but it is not clear which one is the predominant way for transmission (Banatvala et al., 1993b; Parsonnet et al., 1999; Song et al., 2000).

***H. PYLORI* ASSOCIATED DISEASES**

1. Gastritis

After the acute phase, most of the infected individuals are not able to clear *H. pylori* from the stomach and gastritis progresses to chronic gastritis. The location of *H. pylori* colonization in the stomach varies between patients. The commonest pattern of gastritis is a mild pangastritis, which does not markedly change gastric physiology. Corpus predominant gastritis is associated with gastric atrophy, hypochlorhydria and an increased risk of gastric cancer. An antral predominant gastritis is associated with high acid secretion and an increased risk of duodenal ulcer disease (Loched and El-Omar, 2007). The natural history of *H. pylori* infection depends on the acid secretion capacity in host. In the case of a low secretory capacity, *H. pylori* is able to colonize a wide niche towards the corpus which leads to corpus gastritis and inhibition of acid secretion, which is mediated by *H. pylori* induced inflammatory cytokines. Consequently, gastritis proceeds to atrophic gastritis, which causes the development of more permanent morphological changes in gastric mucosa (Loched and El-Omar,

2007). In patients with antral-predominant gastritis and a high acid output, gastric metaplasia is likely to develop in the duodenum increasing the risk of peptic duodenal ulcer. However, the use of acid suppressing medicines like PPIs can shift the pattern of gastritis from antral to a corpus-predominant type which induces a higher risk of developing gastric atrophy (Loched and El-Omar, 2007).

After successful eradication therapy, active inflammation rapidly resolves (Valle et al., 1991), but mild chronic gastritis may persist in some individuals for many years (Veijola et al., 2007).

2. Atrophic gastritis

Atrophic gastritis is a late consequence of *H. pylori* infection in about one third or half of infected individuals (Siurala et al., 1985; Kuipers et al., 1995; Valle et al., 1996; Lauwers, 2003). The development of atrophy takes approximately 20 years (Siurala et al., 1968). Loss of mucosal glands leads to failures in secretory functions of the gastric mucosa. Chronic atrophic gastritis has been classically divided into type A and type B. Autoimmune-type A atrophic gastritis is associated with pernicious anaemia caused by malabsorption of vitamin B12, and the atrophy is situated in the corpus and fundus leading to a decrease in the number of glands and a decrease of PGI and acid output (Varis et al., 1979a), whereas serum gastrin levels are elevated (Varis et al., 1979b). In type B atrophic gastritis the atrophy is predominantly located in the antrum mucosa and results in a loss of antral glands and the number of gastrin-secreting G cells and somatostatin-secreting D cells (Sipponen et al., 1990). Intestinal metaplasia is a common finding in atrophic gastritis (Lauwers, 2003). *H. pylori* infection, atrophic gastritis and intestinal metaplasia are well-known risk factors and precursors for gastric cancer (Sipponen et al., 1985; Kuipers et al., 1995; Kuipers et al., 1996; Lauwers, 2003).

2.1. The role of *H. pylori* in atrophic gastritis

The antrum predominant type B atrophic gastritis is in most cases caused by *H. pylori* infection (Kuipers et al., 1995). The role of *H. pylori* in the autoimmune-type A atrophic gastritis is controversial (Varis et al., 1993; Kuipers et al., 1997; Claves et al.,

1998). In recent years, the classical division of type A and type B has been impugned, due to accumulating data suggesting that corpus atrophy is caused by *H. pylori* associated autoimmune process (Valle et al., 1996, Annibale et al., 2002; Bergman et al., 2005). However, the role of *H. pylori* as a trigger in autoimmune gastritis is debatable (Malfertheiner et al., 2007).

The development of atrophic gastritis can be prevented by *H. pylori* eradication, and atrophic changes on gastric mucosa can turn to normal after successful eradication (Kokkola et al., 2002). In advancing atrophic gastritis the helicobacter colonization on the gastric mucosa decreases. The detection of *H. pylori* infection can be underestimated, if only biopsy-based diagnostic methods are used (Kokkola et al., 1998).

2.2. Histological classification of gastritis and atrophy

Mucosal atrophy can be detected by histology or blood tests. Microscopic examination of gastric biopsy specimens reveals the presence, the topographic location and the grade of mucosal atrophy. Gastritis is most commonly classified according to the Sydney System using the elements of morphology, topography and etiology (Price, 1991; Dixon et al., 1996). The morphologic features are graded into mild, moderate and severe. The graded characteristics are chronic inflammation, activity, atrophy, metaplasia, and *H. pylori*. The microscopical criteria for mild, moderate, and severe atrophy are a loss of the glands less than 30%, 30-60%, and more than 60%, respectively (Price, 1991). The topographic distribution of chronic gastritis is considered as antrum gastritis, corpus gastritis and pangastritis.

2.3. Serum tests in assessment of atrophy and autoimmunity

Two immunologically distinct groups of pepsinogens exist in the gastric mucosa: PGI is derived from the fundic glands and PGII from the chief and mucus neck cell components of the fundic glands, pyloric glands in the antrum, and Brunner glands in the duodenal bulb. In atrophic gastritis the number of fundic glands declines leading to a decline in serum PGI values without changes in the concentration of serum PGII.

Low serum PGI concentration and low ratio of serum PGI/PGII can be used to detect atrophic changes in the corpus (Samloff et al., 1982).

Gastrin is secreted by the G cells in the antral glands. It activates parietal cells to secrete acid. Several forms of gastrin can be measured in blood and over 90% of circulating gastrin consists of gastrin-34 and gastrin-17. The former is responsible for the basal and continuous acid secretion, and gastrin-17 accounts for the secretion which is stimulated by food ingestion (Miller, 1999). In patients with atrophic corpus gastritis the serum gastrin levels are elevated (Kekki et al., 1991), whereas low serum gastrin-17 reveals atrophic antrum gastritis (Hallsley et al., 1994; Oksanen et al., 2000; Vaajanen et al., 2003).

Parietal cell antibodies (PCA) are detected in about 60% of patients with atrophic gastritis and in over 90% of patients with pernicious anaemia (Whittingham and Mackay, 2005). Antibodies against gastric secreted intrinsic factor, which is produced by parietal cells and contributes to absorption of B12-vitamin, have been found in 50 to 70% of patients suffering from pernicious anaemia (Whittingham and Mackay, 2005).

A test-combination of serum PGI, gastrin-17, and *H. pylori* antibodies seems to establish with high sensitivity and specificity the diagnosis of gastritis and the presence and location of atrophic changes in gastric mucosa (Sipponen et al., 2002).

3. Dyspepsia

Dyspepsia has several definitions. Formerly it has been proposed to classify dyspepsia into symptom subgroups of ulcer-like dyspepsia, dysmotility-like dyspepsia, reflux-like dyspepsia, and unspecified dyspepsia (Talley et al., 1992). According to the Rome II criteria, dyspepsia is defined as upper abdominal pain or discomfort (Talley et al., 1999). In a random sample of 4581 Danes aged 30-60 years, the prevalence of dyspepsia within the past 12 months was 54% among men and 47% among women (Kay and Jørgensen, 1994). In Sweden, the prevalence of dyspepsia during the preceding three months was highest among young women decreasing from 26%

among those aged 20-34 years to 14% among those aged 65-79 years, while the prevalence decreased from 17% to 7% among men of corresponding age (Agréus et al., 1994). Depending on the age of the population studied, between 33% and 55% of the patients suffering from dyspepsia are infected with *H. pylori* (Bernersen et al., 1992; Agréus et al., 1995; McColl et al., 1997). Reasons for consulting a physician due to dyspepsia are multifactorial, but *H. pylori* infection, lower socioeconomic status, frequent or severe symptoms, and increasing age are reported to be independent predictors of seeking medical help (Ford et al., 2007). Also in Finland increasing age has been connected with the consultation frequency regarding dyspepsia (Heikkinen et al., 1996).

A ‘test-and-treat’ strategy study on uninvestigated dyspepsia in Canada demonstrated a significant symptom benefit at 12 months with *H. pylori* eradication (Chiba et al., 2002). The effect of eradication treatment on symptoms and related health costs of *H. pylori* infected dyspepsia patients has been actively studied. The benefit achieved in symptoms and health costs seems to correlate to the length of the follow-up, since significant reductions in total dyspepsia-related health care costs were reported after 10 years follow-up (Ford et al., 2005). It has been calculated that about 10 % of the patients suffering from functional dyspepsia could benefit from successful *H. pylori* eradication treatment and 14 patients would have to be treated to cure one case (Moayyedi et al., 2005), and that 30 individuals with *H. pylori* would have to be treated to prevent one person consulting their doctor for dyspepsia during two years follow-up (Lane et al., 2006). According to the latest European guidelines, *H. pylori* ‘test and treat’ is appropriate for patients with uninvestigated dyspepsia (Malfertheiner et al., 2007).

4. Peptic ulcer

Soon after the detection of *H. pylori* the bacterium was associated with duodenal and gastric ulcers (Marshall and Warren, 1983; Marshall and Warren, 1984). In Western countries, duodenal ulcers are about four-fold more common than gastric ulcers, while elsewhere the gastric ulcers are more common (Kusters et al., 2006). The antral predominant form of active chronic gastritis is associated with duodenal ulcer disease

(Dixon, 2000). The role of *H. pylori* in peptic ulcer disease is generally accepted (Blaser, 2004) and eradication therapy is strongly recommended (NIH Consensus Development Panel, 1994). Approximately 95% of the duodenal ulcers are caused by *H. pylori* infection and the recurrence of the ulcers is prevented by successful eradication of *H. pylori*, while about 70% of the gastric ulcers, commonly caused also by NSAIDs, is associated with *H. pylori* infection (Ford et al., 2004). The relationship between *H. pylori* infection and NSAIDs in gastroduodenal ulcers is complex, since *H. pylori* and NSAIDs independently and significantly increase the risk of peptic ulcer bleeding by 1.8 and 4.9-fold, respectively, and the risk is increased by 6.1-fold when both the factors are present (Huang et al., 2002). In Finland, the prevalence of *H. pylori* associated peptic ulcer disease has declined due to decreasing prevalence and active treatment of the infection (Veijola et al., 2005a; Koivisto et al., 2005).

5. Mucosa associated lymphoid tissue lymphoma

H. pylori is associated with the development of the majority of primary low grade gastric MALT lymphomas (Wotherspoon et al., 1991; Lochhead and El-Omar, 2007). In this rarely occurring disease, 62% of the patients with a low grade gastric MALT lymphoma have demonstrated a complete remission after successful eradication (Malfertheiner et al., 2007).

6. Gastric cancer

H. pylori infection has been classified as a first class carcinogen for gastric cancer (International Agency for Research on Cancer, 1994) and it is estimated that 60-90% of gastric cancers are associated with *H. pylori* (Malfertheiner et al., 2005). In Finland, declining local *H. pylori* prevalence rates seemed to predict the declining local incidences of gastric cancer after a median latency of 20 years (Rehnberg-Laiho et al., 2001). Of the common patterns of gastritis, the corpus predominant gastritis is associated with hypochlorhydria, gastric atrophy and intestinal metaplasia widely accepted to be precursors for gastric cancer, and an increased risk of gastric cancer (Kuipers et al., 1995; Valle et al., 1996; Lauwers, 2003; Lochhead and El-Omar, 2007).

It has been suggested that *H. pylori* eradication for gastric cancer prevention in populations at risk should be evaluated (Forman and Graham, 2004; Malfertheiner et al., 2007). An important determinant in cancer prevention is the so-called 'point of no return', at which the premalignant morphological changes in gastric mucosa are irreversible. In reports on the effect of *H. pylori* eradication on the development of gastric cancer, the development of cancer was prevented only in patients, who had no atrophic gastritis, intestinal metaplasia or dysplasia before eradication therapy (Wong et al., 2004).

7. Extragastric diseases

The possible role of *H. pylori* infection in extragastric diseases has been actively studied. The latest European guidelines for the management of *H. pylori* infection recommend searching and treating for *H. pylori* infection in patients suffering from iron deficiency anaemia or idiopathic thrombocytopenic purpura (Malfertheiner et al., 2007), whereas *H. pylori* has no proven role in other extragastric manifestations.

DIAGNOSTIC METHODS FOR *H. PYLORI* INFECTION

The accuracy of a diagnostic test is strongly dependent on the prevalence of the infection (Leodolter et al., 2001; Oksanen et al., 2001). In areas of low *H. pylori* prevalence, the diagnostic test used can demonstrate a lower positive predictive value resulting in an increased number of false positive test results. No single test can be considered a gold standard for the detection of *H. pylori* infection, since all tests have their limitations, which depend on the clinical situation and the local facilities (Cutler et al., 1995).

There are several invasive and non-invasive methods available for the diagnosis of *H. pylori* infection. The invasive methods necessitate taking of gastric biopsies with the aid of gastroscopy. The clinical situation of the patients determines whether a

gastroscopy is needed or whether a non-invasive method is more preferable (Malfertheiner et al., 2002; Pikkarainen et al., 2002). Gastroscopy is not needed for the diagnosis of *H. pylori* infection but if the patient is gastroscopied for clinical reasons, gastric biopsies can be used for the diagnosis of *H. pylori* as well.

1. Invasive methods

1.1. Rapid urease test

In rapid urease tests (RUTs), the urease of *H. pylori* splits the urea in a gel or liquid and produces ammonia, which causes a rise in pH. This is demonstrated by a change in colour from yellow to red in test medium with the aid of an indicator within 3 hours with the newer tests (Marshall et al., 1987; Tseng et al., 2005). There are both in-house and commercial RUTs available. The accuracy for RUTs in detecting *H. pylori* is considered high demonstrating sensitivities and specificities exceeding 90% (Laine et al., 1996; van Keeken et al., 2006).

1.2. Histology

The silver staining of antral biopsies has been shown to be most accurate in detecting *H. pylori* infection in histology, compared to serological tests and UBTs (Cutler et al., 1995). In addition to insensitive haematoxylin-eosin staining, the use of highly sensitive modified Giemsa staining is recommended for clinical use for the detection of *H. pylori* (Madan et al., 1988; Dixon et al., 1996).

The histological findings are classified by the Sydney System (Price, 1991; Dixon et al., 1996), in which the presence of *H. pylori* is graded from 0 to 3. According to this system, two biopsies from both antrum and corpus are needed for histology. The diagnostic and grading criteria suggested by the Sydney System have been well accepted by histopathologists (Andrew et al., 1994; el-Zimaity et al., 1996). However, the results are dependent on the experience of the pathologist (Metz et al., 1998), the number of biopsies taken (van IJzendoorn et al., 2005), and the density of *H. pylori* in gastric mucosa (Testoni et al., 2002). With an experienced pathologist, the sensitivity

and specificity of histological examination in detecting *H. pylori* infection were 94% and 100%, respectively, as compared to a combination of gastritis and elevated *H. pylori* antibodies (Oksanen et al., 1998).

1.3. Culture and antimicrobial susceptibility

The sensitivity of culture is usually lower than that of other methods and is influenced by the transport medium, time, and the experience of the laboratory personnel (de Boer, 1997; Pérez-Pérez, 2000). *H. pylori* is relatively slow-growing and culture plates should be incubated for 12 days (Rautelin et al., 1997). Following an incubation time of 12 days, the sensitivity and specificity of culture in detecting *H. pylori* infection were 77% and 100%, respectively, as compared to a combination of gastritis and elevated *H. pylori* antibodies (Oksanen et al., 1998).

Culture offers an opportunity for testing the antimicrobial susceptibility, which is clinically important, since *H. pylori* develops resistance against metronidazole and clarithromycin which are commonly used in eradication treatments. It is also possible to further characterize the *H. pylori* strains isolated, although the detection of virulence factors is not recommended in clinical practice. In clinical use, culture of *H. pylori* and antimicrobial susceptibility test are indicated after one or two failed eradication treatments (Mégraud and Lehours, 2007).

1.4. Molecular methods

The polymerase chain reaction (PCR) is a technique in which predetermined fragments of bacterial DNA are amplified. It is seldom used in clinical practice. For scientific purposes, it enables to determine virulence genes like *cagA* and fingerprinting for epidemiological surveys (Schwarz et al., 1997).

2. Non-invasive methods

2.1. Blood tests

2.1.1. Enzyme immunoassay for *H. pylori* antibodies

EIA is the most commonly used serological test for detecting antibodies to *H. pylori*, since it is reliable, simple, and inexpensive. In EIA-based tests the antigen is adsorbed onto plastic support, usually a 96-well polyvinyl or polystyrene microtitre plate. Diluted serum is incubated with the immobilized antigen and antigen-antibody-complexes are formed. The complexes are detected using an enzyme-conjugated (anti-human) antibody. The amount of enzyme, which is representing the amount of bound antibodies, is visualized using a chromogenic enzyme substrate.

The special role of EIA serology has recently been emphasized compared to other diagnostic tests; the usefulness of this method is not affected by prior use of PPIs, ulcer bleeding, or the diminished number of helicobacters on the gastric mucosa (Malfertheiner et al., 2007). With EIA it is possible to measure *H. pylori* antibodies of different immunoglobulin classes and to make quantitative determinations. Quantitative serological assays can be used both in the diagnosis of the infection and in the follow-up after eradication treatment. Using serology as a follow-up method after treatment requires taking a pre-treatment serum, a post-treatment serum four to six months after therapy and testing the samples in parallel in the same assay to determine the decline of antibody titres. The criterion for successful eradication is an antibody titer decline of at least 40% four to six months after treatment (Kosunen et al., 1992; Lerang et al., 1998; Rautelin and Kosunen, 2004).

There are several commercial serological kits available (Lahei et al., 1998). The best commercial kits have shown sensitivities and specificities greater than 95%; the sensitivity and specificity of the commercial tests has varied between 60 and 100% (Feldman et al 1995; Lahei et al 1998). The best quantitative Finnish commercial and in-house EIA-tests detecting *H. pylori* IgG antibodies show sensitivities of 99% and specificities of 97-98% (Oksanen et al., 1998) and of these tests, the commercial

Pyloriset EIA-G (Orion Diagnostics, Espoo, Finland), which is widely used in Finland, proved to have the best accuracy in a comparison of several commercial EIA-assays testing the same sera in several laboratories in Europe (Feldman et al., 1995).

The serologic tests may, however, demonstrate a lower performance if the population has been actively treated for *H. pylori* infection, since after successful *H. pylori* eradication therapy the antibodies decline slowly to normal levels. The antibodies declined below the cut-off level only in 28% of successfully treated patients after one year (Lerang et al., 1998), and after five years, 33% of individuals still demonstrated elevated antibody levels after successful eradication therapy (Veijola et al., 2007). It has been recommended that antibody assays should be evaluated locally (Feldman et al., 1995; Hernbrink and van Doorn., 2000). In addition to the properties of the serology kit used, the reference method determining active *H. pylori* infection, the selection of patients and variations in the antigenic properties of the bacterial strains can explain the variation in sensitivity and specificity between different EIA tests (Hernbrink and van Doorn, 2000; Laheij et al., 1998).

2.1.2. Near patient tests

Point-of-care tests are mostly whole blood tests. They are not currently recommended because of low accuracy (Malfertheiner et al., 2007).

2.1.3. Immunoblot assay

In immunoblot assays antigens are separated by electrophoresis according to their molecular weight and transferred onto absorbent paper in order to react with specific antibodies. The antibodies are detected by a conjugate and substrate as in EIA. Immunoblots are not used in clinical practice but could be used in special circumstances for instance to detect CagA antibodies (Nilsson et al., 1997).

2.2. Urea breath test

The UBT is based on the ability of *H. pylori* on the gastric mucosa to hydrolyse ingested isotope-labelled urea by urease enzyme which produces isotope-labelled CO₂. The labelled CO₂ diffuses then into the blood and is excreted by the lungs and can be measured in the exhaled air. Both radioactive ¹⁴C and non-radioactive ¹³C-labelled urea have been used (Marshall and Surveoyr, 1988; Graham et al., 1987). Measurement of the ¹³C isotope, which is more commonly used, is performed by isotope ratio mass spectrometry, non-dispersive isotope-selective infrared spectrometry or a laser assisted ratio analyser.

UBT has proved to be highly accurate both in diagnosing *H. pylori* infection and in the follow-up after treatment demonstrating a sensitivity and specificity exceeding 95% and is widely used (Gisbert and Pajares, 2004a). However, preceding antimicrobial or PPI-treatment, bleeding ulcer, and conditions associated with low bacterial density on gastric mucosa can lead to false negative test results (Malfertheiner et al., 2007).

2.3. Stool antigen tests

Tests detecting *H. pylori* antigens in stool samples are based on monoclonal or polyclonal antibodies. It is necessary to store the stool samples at -20°C until analysed. In a systematic review of 89 studies (Gisbert and Pajares, 2004b), the sensitivity and specificity of stool antigen tests were 91% and 93%, respectively. The monoclonal tests have performed well both in primary and post-therapy diagnosis (Veijola et al., 2005b).

2.4. Other tests

EIA-based salivary antibody tests have generally demonstrated quite low diagnostic accuracy (Kabir, 2003) and they have no current role in patient management (Malfertheiner et al., 2007). The commercial tests for detecting *H. pylori* antibodies in urine are based on EIA (Katsuragi et al., 1998) or rapid immunochromatographic methods (Wu et al., 2001). The EIA-based urine test (Urinelisa) showed only modest

accuracy (sensitivity 63-89% and specificity 68-97%), when evaluated in multicenter studies in children (Mégraud et al., 2005) and in adults (Leodolter et al., 2003). An immunochromatographic test (Rapirun) showed a sensitivity of 82% and a specificity of 83% in adults (Leodolter et al., 2003). The tests for detecting *H. pylori* antibodies in urine are not regarded useful in patient management, but can be useful in epidemiological studies (Malfertheiner et al., 2007).

3. Diagnosis in special situations

3.1. Children

The sensitivity of serological tests based on IgG antibody detection is low in children younger than six years, but the decline in antibody titres is useful in confirming the success of eradication treatment in children (Kolho et al., 2002). UBT has proved to be accurate in children although false positive result may occur in young children (Kolezko and Feydt-Schmidt, 2001). The performance of stool antigen tests has proved to be excellent in young children demonstrating accuracy exceeding 95% (Kolho et al., 2006).

3.2. The elderly

The prevalence of serious diseases increases by age. Consequently, the management of dyspepsia in the elderly easily includes endoscopic examination with gastric biopsies, which allows detection of macroscopic and microscopic abnormalities. The detection of *H. pylori* is more challenging with the elderly (Salles and Mégraud, 2007), since the prevalence of atrophic gastritis is higher in this age-group than among younger individuals, which can lead to false negative test results in histology due to a lower bacterial density on mucosa. Serology is the method of choice in detecting *H. pylori* infection in the case of atrophic gastritis or other conditions with a low density of bacteria. However, there is a possibility of false positive test results, due to slowly declining antibody levels after a cured infection (Veijola et al., 2007). UBT is regarded to obtain the best performance in the elderly (Salles and Mégraud, 2007).

3.3. Bleeding peptic ulcer

Successful *H. pylori* eradication prevents the recurrence of the ulcers among infected subjects (Ford et al., 2004). *H. pylori* eradication also prevents effectively rebleeding in *H. pylori* infected individuals, while the risk of rebleeding is approximately 50% at 10 years, if the patient is left untreated after ulcer healing (Gisbert et al., 2004c). In the case of ulcer bleeding, the detection of *H. pylori* is complicated, since PPIs are regularly used which with acute ulcer bleeding impairs the sensitivity of biopsy-based methods and UBT for detecting *H. pylori*-infection (Malfertheiner et al., 2007). In this special situation, the role of serology is emphasized as an accurate method to diagnose *H. pylori* infection (Malfertheiner et al., 2007).

3.4 Atrophic gastritis

Patients with atrophic corpus gastritis have often positive helicobacter serology, but their histologic findings often remain helicobacter negative (Karnes et al., 1991; Testoni et al., 1996; Kokkola et al., 2000). Along with advancing atrophic gastritis the helicobacter colonization on the gastric mucosa decreases. The diagnosis and prevalence of *H. pylori* infection can remain underestimated, if only biopsy-based diagnostic methods are used. Due to advanced gastric atrophy, many culture, histology and breath test negative subjects may still be infected which has been shown by rapidly falling antibody titres in these patients after eradication therapy (Kokkola et al., 1998). Serology is sensitive in detecting *H. pylori* in the case of atrophy (Malfertheiner et al., 2007), but the specificity may be lower, since this method does not differentiate between a current and a past infection; after successful *H. pylori* eradication therapy the antibodies decline slowly to normal levels (Veijola et al 2007).

GUIDELINES FOR THE MANAGEMENT OF *H. PYLORI* INFECTION

1. European guidelines

The first European Guidelines, ‘Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht Consensus Report’ was published in 1997 (EHPSG, 1997). It summarized the latest knowledge in the area provided by 63 experts from 19 European countries. The update of the Guidelines, ‘Current concepts in the management of *Helicobacter pylori* infection – The Maastricht 2-2000 Consensus Report’ was published in 2000 (Malfertheiner et al., 2000), and the latest update, ‘Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report’ was published in 2007 (Malfertheiner et al., 2007). Each of the guidelines emphasized special viewpoints, although the themes about ‘how to test’, ‘whom to test’ and ‘how to treat’ were discussed in each report.

1.1. The first Consensus Report

Special themes were: the role of primary care physician, patient management level, and public health issues. Primary care physicians were advised to diagnose *H. pylori* infection using UBT or locally validated serology in dyspeptic patients younger than 45 of age and without alarming symptoms, while other dyspeptic patients were referred to a specialist. A close collaboration and interaction between primary care physicians (GPs), gastroenterologists and microbiologists was recommended (EHPSG, 1997).

Strongly recommended indications for eradication therapy were: peptic ulcer disease, bleeding peptic ulcer, low-grade gastric MALT lymphoma, gastritis with severe abnormalities, and earlier resection for gastric cancer. Advisable indications were: functional dyspepsia after full investigation, family history of gastric cancer, long-term treatment with PPIs for gastro-oesophageal reflux disease (GORD), planned or existing NSAID therapy, gastric surgery for peptic ulcer, and patient’s wishes. Uncertain indications were: prevention of gastric cancer, treatment of asymptomatic

subjects and an extra-alimentary tract disease. The recommended treatment regimens consisted of triple therapies with metronidazole/tinidazole and clarithromycin, amoxicillin and clarithromycin, or amoxicillin and metronidazole, all combined with PPI.

1.2. The second Consensus Report

Special themes were: *H. pylori* diagnosis and eradication in primary care, indications for eradication therapy, and prevention of cancer and other *H. pylori* related gastroduodenal pathologies. The GPs were advised to diagnose the infection with UBT or stool antigen test and always verify the success of the eradication therapy. A 'search-and-treat' strategy was recommended for peptic ulcer patients on long-term or intermittent antisecretory therapy (Malfertheiner et al., 2000).

Strongly recommended indications included (in addition to those described in the first Consensus Report) also atrophic gastritis, post-gastric cancer resection, gastric cancer in first degree relatives, and patient's wishes (after full consultation). *H. pylori* eradication was considered not to be associated with GORD development. *H. pylori* eradication was considered to lead to long-term symptom improvement in a subset of patients suffering from functional dyspepsia. Bismuth subcitrate based quadruple therapy of seven days was recommended for a second course of treatment after a treatment failure.

1.3. The third Consensus Report

The special themes were: Indications and contraindications for eradication, focusing on dyspepsia, NSAIDs, GORD, diagnostic tests and treatment of infection, and prevention of gastric cancer (Malfertheiner et al., 2007).

1.3.1. Dyspepsia, GORD, NSAIDs, and extraintestinal diseases

It was recommended that *H. pylori* eradication is appropriate for patients infected with *H. pylori* and investigated non-ulcer dyspepsia, and *H. pylori* 'test-and-treat' is appropriate for patients with uninvestigated dyspepsia. The effectiveness of *H. pylori*

‘test-and-treat’ was considered low in populations with a prevalence of < 20% and empirical acid suppression was considered an equivalent option. It was stated that *H. pylori* eradication does not cause GORD, and *H. pylori* testing and treating should be considered for patients receiving long-term maintenance treatment with PPIs. *H. pylori* eradication was recommended in naïve users of NSAIDs and in the case of bleeding in patients receiving long-term aspirin treatment. Related to extraintestinal diseases it was recommended to search for and treat the infection in patients with unexplained iron deficiency anaemia and in patients with idiopathic thrombocytopenic purpura.

1.3.2. Special role of serology

The consensus report emphasized the special role of serology as a diagnostic test in situations where other tests could be false negative, for example in bleeding ulcers, gastric atrophy and other conditions of low bacterial density, MALT lymphoma, and recent or current use of PPIs. However, serology-based point-of-care tests and the detection of specific *H. pylori* antibodies in urine or saliva have no current role in the patient management, according to the report.

It was recommended to confirm *H. pylori* eradication at least four weeks after treatment by UBT or a laboratory stool antigen test using monoclonal antibodies. Culture and antimicrobial sensitivity testing was recommended to be performed routinely before clarithromycin based treatment, if primary resistance is greater than 15-20%, and after two treatment failures.

1.3.3. Eradication treatment regimens

A triple therapy was recommended including PPI (standard dose) b.i.d., clarithromycin (500mg) b.i.d., amoxicillin (1g) b.i.d. or metronidazole (400 or 500mg) b.i.d. for 14 days or for seven days if local studies show that it is effective. In populations with less than 15-20% clarithromycin resistance, triple therapy including PPI, clarithromycin, and amoxicillin/metronidazole was recommended. In populations with less than 40% metronidazole resistance, the use of PPI-clarithromycin-metronidazole is preferable. Quadruple treatments were considered as an alternative

first choice treatment. For a second course of treatment, bismuth-containing quadruple treatments were recommended, if available, or triple therapies including PPI, amoxicillin or tetracycline, and metronidazole, if bismuth is not available. For rescue treatment antimicrobial susceptibility testing should precede the choosing of a treatment regimen, according to the report.

1.3.4. The relation between *H. pylori* and gastric cancer

It was stated that the global burden of gastric cancer is increasing, predominantly in developing countries and *H. pylori* is the most common risk factor for human non-cardiac gastric cancer. It was stated that the risk for gastric cancer development depends on bacterial virulence factors and hosts genetic factors, and also environmental factors contribute to the risk. Eradication of *H. pylori* prevents the development of pre-neoplastic changes of the gastric mucosa and has the potential to reduce the risk of gastric cancer development, and the optimal time for eradication is before pre-neoplastic conditions (atrophy, intestinal metaplasia) are present, probably in early adulthood, according to the report. *H. pylori* eradication for gastric cancer prevention in populations at risk was recommended to be evaluated and considered.

2. Finnish guidelines

The first Finnish recommendation for the management of *H. pylori* infection was published in January 1996 and providing advice as to how to diagnose *H. pylori* infection, whom to treat, and what treatment regimens should be used (Färkkilä et al., 1996). The second Finnish recommendation was published in March 2000 (Suomen gastroenterologiyhdistys ry, 2000).

2.1. The first Finnish guidelines

According to the first guidelines, it was possible to detect *H. pylori* by histology, by culture or RUT of the biopsies obtained during gastroscopy, or by non-invasive methods using serology or UBT, if available. Strongly recommended indications for eradication treatment were active or nonactive peptic ulcer disease, while advisable or

uncertain indications were the use of NSAIDs and functional dyspepsia with severe symptoms after gastroscopy, according to the guidelines (Färkkilä et al., 1996).

The recommended treatment regimens included amoxicillin (1g) b.i.d. (or tetracycline (500mg) q.i.d. for those allergic to amoxicillin), metronidazole (400mg) t.i.d. and PPI (standard dose) b.i.d. for seven days. The second course of treatment included tetracycline (500mg) q.i.d. or amoxicillin (1g) b.i.d., metronidazole (400mg) t.i.d., bismuth subcitrate (240mg) and PPI (standard dose) b.i.d. for seven days, or amoxicillin (1g) b.i.d. or tetracycline (500mg) q.i.d., clarithromycin (250mg) b.i.d. and PPI (standard dose) b.i.d. for seven days. The success of treatment was recommended always to verify performing gastroscopy with biopsies in gastric ulcers, and by using UBT, serology, or gastroscopy in other patients.

2. 2. The second Finnish guidelines

According to the 2000 guidelines, if gastroscopy is indicated for clinical reasons, gastric biopsies (two biopsies from antrum and two from corpus for histologic examination) can be used for the diagnosis of *H. pylori* infection. An active ulcer or another condition requiring immediate eradication treatment indicates taking additional biopsies for RUT, and two or more previous courses of eradication treatment indicates taking additional biopsies for culture. If gastroscopy is not needed, ¹³C-UBT, quantitative serology or a stool antigen test is the method of choice for the diagnosis of *H. pylori* infection. It is recommended to verify the success of treatment by UBT or quantitative serology (Suomen gastroenterologiyhdistys ry, 2000).

The indications for eradication therapy are almost similar to those in the third European Consensus Report. The Finnish indications include peptic ulcer disease with or without ulcer bleeding, gastric surgery for peptic ulcers, gastric surgery for localized gastric cancer or premalignant lesion, MALT lymphoma, atrophic gastritis, dysplasia, gastric cancer in a close relative, new dyspepsia, functional dyspepsia, and the use of NSAIDs. Screening for *H. pylori* infection among asymptomatic individuals or in extragastric diseases is not recommended, according to the Finnish guidelines.

The recommended treatment regimen for adults consists of PPI (standard dose) or ranitidine-bismuth (400mg), amoxicillin (1g) and clarithromycin (500mg) b.i.d. for seven days, or PPI (standard dose) or ranitidine-bismuth (400mg), metronidazole (400mg), and clarithromycin (250mg) b.i.d. for seven days, or ranitidine-bismuth (400mg) b.i.d., metronidazole (250mg) q.i.d. or (500mg) t.i.d., and tetracycline (500mg) q.i.d. for seven days. It is not recommended to use metronidazole, if the local metronidazole resistance is over 30%. After two failed treatment regimens, culture and testing for the antimicrobial susceptibility are indicated, according to the guidelines.

The second Finnish guidelines were updated in online in 2002 (<http://www.terveysportti.fi>). According to the updated guidelines, the recommended treatment regimen for adults consists of PPI (standard dose), amoxicillin (1g) and clarithromycin (500mg) b.i.d. for seven days, or ranitidine-bismuth (400mg), amoxicillin (1g) and clarithromycin (500mg) b.i.d. for seven days. The recommended second course of treatment includes ranitidine-bismuth (400mg) b.i.d., metronidazole (400mg) t.i.d. and tetracycline (500mg) q.i.d. for seven days. After two failed treatment regimens, culture and testing the antimicrobial susceptibility are indicated.

SCREENING AND TREATING FOR *H. PYLORI*

1. Is *H. pylori* infection appropriate for screening?

In general, an ideal screening programme should have following prerequisites for the disease (WHO, 1968): 1) The treatment improves the prognosis if it is given at an early state of the disease. 2) The disease is common enough and the consequences are significant (mortality, invalidity, morbidity). 3) It is possible to detect the disease at an early stage. 4) There is a long delay from the beginning of the disease to the clinical symptoms. Infection with *H. pylori* is mostly acquired in childhood. It causes life long bacterial infection on the gastric mucosa and causes a progressive damage of

the mucosa which can lead to peptic ulcer disease or premalignant and malign conditions. It usually does not heal without specific antimicrobial treatment. The disease prerequisites for an ideal screening programme are fulfilled, since *H. pylori* eradication improves dramatically the prognosis of peptic ulcer disease and prevents the development of gastric cancer, if it is given at early stage, before the outcome of premalignant conditions, and it also improves the prognosis in some of the persons suffering from dyspepsia. *H. pylori* infection is one of the most common infections in the world, although the prevalence of the infection is rapidly declining in the Western world and the consequences are severe. It is possible to diagnose the infection at early stage, and there is usually a long delay to the clinical outcomes.

Furthermore, an ideal screening programme should have following prerequisites for the test (WHO, 1968): 1) The test demonstrates a high sensitivity and specificity. 2) The test is cheap, simple, and is suited to large populations. 3) The test is safe and acceptable. 4) The test is easy to repeat. 5) The possible further studies should not be expensive or unsafe. Locally validated *H. pylori* antibody assays with a high accuracy fulfil the test prerequisites for an ideal screening programme, since they are cheap, simple, safe, acceptable, suitable also for large populations, and easy to repeat. More expensive urea breath tests are also suitable for this purpose. If needed, an UBT, stool test or gastroscopy already common in clinical use can be utilized for possible further testing.

2. Recruitment for clinical trials

Recruitment of an adequate number of participants is essential to achieve a successful completion of a clinical trial. Sometimes studies report the need to extend the recruitment period to achieve a large number of participants to reach statistically significant results (Lovato et al., 1997). It is important to plan carefully and carry out a pilot study before the trial and if needed alter the existing plans for requirement strategies according to the results of piloting. Recruitment of diverse populations such as ethnic minorities, men, the elderly and individuals aged less than 45 years have appeared to be challenging according to previous reports (Slattery et al., 1995; Eachus

et al., 1996; Lovato et al., 1997). In addition, material deprivation has been associated with lower participation rates (Lane et al., 2002).

In recruitment the using of national population registries, national disease related registries, local databases containing lists of patients or other databases is utilized. Screening for potential participants at an occupational site offers a possibility to easily contact a large number of individuals (Lovato et al., 1997). Invitation by mail is a relatively low-cost approach for contacting large numbers of potential participants (Bjornson-Benson et al., 1993). Personalized letters and a coordinated media-campaign have improved the efficiency and effectiveness of this strategy (Bjornson-Benson et al., 1993). Recruiting participants using announcements in media is also possible.

3. Reasons for non-compliance in screening

In considering the effectiveness of a screening programme, it is important to take into account non-compliance. According to a systematic review, barriers to participation in randomized controlled trials were divided into clinician barriers and patient barriers (Ross et al., 1999). The clinician barriers included: time constraint, lack of staff and training, worry about the impact on the doctor-patient-relationship, concern for patients, loss of professional autonomy, difficulty with the consent procedure, lack of rewards and recognition, and an insufficiently interesting question, while the patient barriers included: additional demands of the trial, patient preferences, worry caused by uncertainty, and concerns with information and consent. To overcome the barriers to recruitment, the trial should address an important research question, data collection should be as simple as possible, and trials should be carefully planned and piloted (Ross et al., 1999).

Possible reasons for non-compliance with screening for *H. pylori* reported were that individuals invited were too busy (work commitments), they had family commitments, the appointment time was inconvenient, the individuals were on holiday or away from home, there was no recall of receiving an invitation letter, the letter was not read or not fully understood, there were no symptoms, the individuals

were not interested, they forgot about the appointment or had another illness/disability (Stone MA et al., 1998a). Material deprivation and age was associated with a low participation rate in screening so that the participation rate increased by age (Laine et al., 2002).

4. Population screening and treating for *H. pylori*

Randomized controlled trials (RCT) generally provide more reliable information than observational studies, and are widely accepted as the most powerful research method for minimising bias when evaluating health technologies. A systematic review of RCTs with good methodological quality and homogeneity, as well as an individual randomized controlled trial with narrow confidence interval are regarded to offer the best scientific evidence, which is not reached by non-controlled studies (Malfertheiner et al., 2007). However, the participation rate in RCTs remains often low among other reasons due to the possibility of receiving placebo (Ross et al., 1999; Lane et al., 2002).

There are reports on population-based ‘screen-and-treat’ trials, in which study population consists of randomly selected general population or subjects registered with primary care centres. The trials can be classified to RCTs, controlled trials, and other population trials.

4.1. Randomized controlled population trials

In a community screening programme for *H. pylori* in Leeds in 1994 a total of 32,929 randomly selected individuals aged 40-49 years and listed in 36 primary care centres were invited to the study. Participants attended their local practice and their *H. pylori* status was examined by UBT. Eradication was determined with a UBT six months and two years after the first visit. A total of 8407 individuals participated in screening and were evaluable (25.5% of those invited), 2329 tested positive (27.7% of those evaluable), and 1161 individuals were randomized to *H. pylori* eradication therapy including omeprazole (20mg), clarithromycin (250mg) and tinidazole (500mg) twice daily for seven days, and 1163 individuals to identical placebo. *H. pylori* eradication

rates were 73% per protocol and 61% in intention-to-treat analysis in the treatment-group (Moayyedi et al., 2000a; Moayyedi et al., 2000b).

The Bristol *Helicobacter* project started in 1996 as a double-blind placebo-controlled trial of the effect of *H. pylori* eradication on symptoms of dyspepsia. The target population of 27,536 individuals aged 20-59 years and registered with seven primary care centres in Bristol, UK and the surrounding areas was invited to undergo a UBT (Lane J et al., 2002). Eradication was determined with a UBT six months after treatment. Of the participants, 23.5% had dyspepsia on study entry. A total of 10,537 individuals (38.3% of those invited) participated in screening, 1636 tested positive (15.5% of those tested), and 1558 (95.2% of those tested positive) were randomized to *H. pylori* eradication therapy including ranitidine-bismuth citrate (400mg) and clarithromycin (500mg) twice daily for 14 days, or placebo (Lane et al., 2002). *H. pylori* eradication rates were 91% per protocol and 84% in an intention-to-treat analysis (Harvey et al., 2004).

4.2. Controlled population trials

In Odense, Denmark, a population-based intervention study was started to assess the effect of *H. pylori* screening and eradication in general population on the prevalence of dyspepsia, incidence of peptic ulcer disease and possible savings in health care costs, and on the quality of life (Wildner-Cristiansen et al., 2003). The target population of 80,643 individuals aged 40-64 years and living in Odense and the surrounding municipalities was identified by their civil registration numbers in the county's population register, and 21,998 individuals were selected on the basis of addresses and proportional to the number of persons in each 5-year age group in the background population. The selected individuals were randomized by computer for an intervention group, which was offered *H. pylori* screening and eradication, and a control group by a one-to-one randomization. The screening was two-staged as a positive in-office whole-blood-test-result was confirmed by UBT. The eradication treatment included omeprazole (20mg), clarithromycin (500mg) and amoxicillin (1g) twice daily for 7 days.

H. pylori eradication was confirmed in 20% of the treated subjects by a second UBT at least one month after eradication treatment. The prevalence of dyspepsia and the quality of life were assessed using mailed questionnaires and information on the use of endoscopies and the use of prescription medication was obtained from various health registers. The participation rate was 63% and 1008 tested *H. pylori* positive (17.5% of the 5749 participants). The *H. pylori* eradication was successful in 95% of the tested subjects (Wildner-Christiansen et al., 2003).

4.3. Other community based trials

In Leichestecher, UK, a general population sample of 4015 subjects aged 21-55 years was offered a serological screening test for *H. pylori* through their general practitioners (Stone et al., 1998b). *H. pylori*-positive subjects were offered a prescription for eradication treatment and an information pack designed to encourage good compliance. Success of treatment was assessed by UBT. The participation rate in screening was 39%. Of the 235 (15%) subjects who were *H. pylori*-positive, 186 (79%) were treated. Eradication was confirmed in 170 (95%) of the 179 subjects who had a UBT. Assuming a 15% overall prevalence of *H. pylori* infection it was estimated that *H. pylori* was eradicated in 29% of those infected in the target population of 4015 subjects. Effectiveness of the programme was limited most strongly by acceptance of the screening test (Stone et al., 1998b).

In Matsu Island in Taiwan, with a population of 8732 in 2003 according to Britannica Online Encyclopaedia (<http://www.britannica.com>), a community-based study of *H. pylori* therapy using the strategy of 'test, treat, retest, and retreat initial treatment failures' was started in 2004 (Lee et al., 2006). The target population comprised of 3700 native residents of Matsu Island aged 30 years or older who were registered in the Island's population list. The report does not define exactly whether the study population comprised of all or a part of the residents of the Island in the selected age-group.

Residents of the Island have a high prevalence of *H. pylori* infection, and the annual mortality rate for gastric cancer is 33 deaths per 100,000 population (Lee et al., 2006). To ascertain the presence of pre-cancerous lesions and gastric cancer, the screening was two-staged using UBT in the first stage and an endoscopy for those who tested

positive in UBT in the second stage. Eradication success was confirmed by UBT. Performing the study in collaboration with a community-based multiple screening programme (Chen et al., 2004) and arranging the UBT-screening sites near shopping centres resulted in a high participation rate of 71,8%. Of the 2658 participants (including 1654 current and 1004 former residents of the Matsu Island), 1651 tested positive in UBT (62.1% of the participants). Endoscopy was performed and an initial eradication treatment including esomeprazole (40 mg) once daily, and amoxicillin (1g) and clarithromycin (500mg) twice daily for seven days was received in 1132 individuals (68.6% of those tested positive). Of them, 939 (82.9%) returned for a second UBT and the eradication rate was 86% per protocol and 71.4% in intention-to-treat-analysis. On the basis of the completed questionnaires of 886 valid reporters, the eradication rates were 88.7% per protocol and 86.9% in intention-to-treat analysis. A total of 105 individuals of those 131 demonstrating a treatment failure with the first treatment were willing to receive retreatment including esomeprazole (40mg) and levofloxacin (500mg) once daily, and amoxicillin (1g) twice daily for ten days. A successful eradication was achieved in 91.4% of the 105 individuals retested with UBT (Lee et al., 2006).

5. Public health perspective

The knowledge and management strategies concerning *H. pylori* infection among GPs has been evaluated. The knowledge about *H. pylori* infection has not been effective at primary care level, since in a worldwide survey, of the 470 randomly selected GPs from 29 countries, 81%, 85% and 61% knew the causal relationship of *H. pylori* with gastric and duodenal ulcers and gastric cancer, respectively, and PPI-based triple therapies were prescribed by 89% of the responders (Huang et al., 2003). *H. pylori* was considered relevant to dyspepsia management among 63% of the GPs, and 66% treated new dyspepsia empirically, 18% chose endoscopic diagnosis and 13% used a 'test and treat' approach (Huang et al., 2003). In a study from England, 44 GP-participants accounting for a population of 262,647 participated and among that population, 6037 (2.3%) of patients were on long-term acid suppressing treatment. Among them, only 131 (10%) of 1306 patients who had peptic ulcer disease had been prescribed eradication therapy (Wright et al., 2001).

PRESENT STUDY

AIMS OF THE PRESENT STUDY

The age-adjusted *H. pylori* seroprevalence rate in the adult population decreased from 51% to 34% from 1973 to 1994 in Vammala, a semiurban community in South-West Finland with a population of about 16,000, before the current use of antimicrobials in the treatment of helicobacter gastritis. Almost all *H. pylori* infected individuals show elevated levels of specific IgG antibodies, and in about two-thirds of subjects the specific IgA antibodies are elevated. Presence of *H. pylori* IgA antibodies has been associated with a CagA-positive infection, which in turn is associated with an increased risk of severe complications of *H. pylori* infection.

The aims of the present work were

1. to accelerate the decline of *H. pylori* gastritis by starting a unique population-based, voluntary 'screen-and-treat' programme in primary health care practice.
2. to examine risk factors for *H. pylori* infection in a Finnish young adult population by a questionnaire.
3. to determine the accuracy of *H. pylori* IgG and IgA antibody tests for adults in different age-groups, with special emphasis on the presence of atrophic gastritis.
4. to study whether an increase in the number of IgA-antibody-positive *H. pylori* patients with age is a cohort phenomenon only, or whether initially IgG-antibody-positive *H. pylori* patients later develop an IgA response, and how the elevated IgA antibody levels change during infection.
5. to determine whether the proportion of subjects infected with CagA⁺ or CagA⁻ *H. pylori* strains has changed as the overall prevalence of *H. pylori* has declined.

SUBJECTS

We started a unique population-based, voluntary '**screen-and-treat**' programme in primary health care (Study I), first as a pilot study in 1994 and since 1996 extending the programme to include all over 6000 inhabitants in Vammala aged 15 to 40 years and 45 years.

A total of 864 subjects were included in **the pilot study** (Study 1). Of these, 284 persons had participated in a health study in 1973 and they had been reinvited in 1994 to local health centre for venepuncture for *H. pylori* antibody testing (see below). Blood samples were obtained from 275 subjects (97% of those 284 invited), including 162 (58.9%) females. The 1973 health study had been organized by the Social Insurance Institution of Finland, which had carried out a population-based health examination including collection and storage of blood samples in 1973. At that time, a random sample of adult population had been selected from the National Population Register by computer. A total of 600 subjects had been invited by mail and 492 had participated. The participation rate had been 84% in 15 to 74-year-old subjects in 1973. From the sera which had been collected in 1973 and stored at -20C°, 408 sera from 213 female and 195 male had been available in 1994, when a follow-up study on the seroprevalence of *H. pylori* infection (Kosunen et al., 1997) had been started (Group A). Of the subjects who had participated in the health study in 1973, 372 were still alive in 1994. Also they had been reinvited by mail to the local health centre for venepuncture and 224 had given a new blood sample for *H. pylori* antibody testing.

Of the total of 864 subjects included in the pilot study, 580 persons were included from a random sample of 600 residents of Vammala selected by computer from the National Population Register in 1994. The random sample of 600 inhabitants had included 50 females and 50 males from each 10-year age group between 15 and 74 years. Of the selected 600 subjects, 594 had been invited by mail to the local health centre for venepuncture for *H. pylori* antibody testing, when the follow-up study on the seroprevalence of *H. pylori* infection had been started (Kosunen et al., 1997). Blood samples were obtained from 504 subjects (84% of the invited) including 262 females and 242 males (Group B). Of the 1994 random sample of 600 inhabitants, 14 individuals had participated in the 1973 health study and were included in the pilot

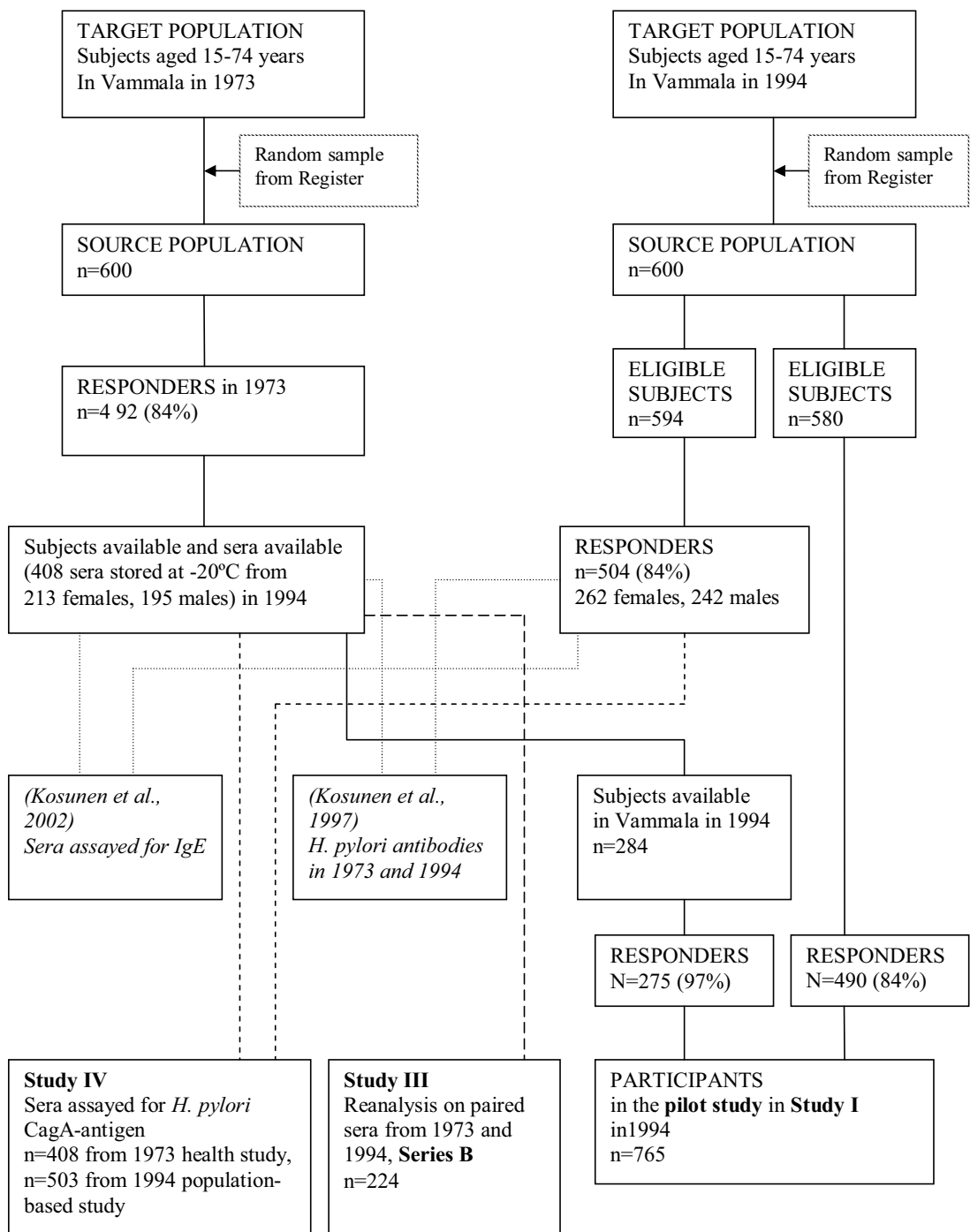


Figure 1. Design of the pilot study in 1994 (Study I) and Studies III and IV. Register, National Population Register.

study in the above described group of 284 persons, and six subjects had been excluded for other reasons. The study design is also presented in Figures 1 and 2. Seroepidemiological findings on *H. pylori* infection and allergen specific IgE concentrations, based partly on this material, have been published previously (Kosunen et al., 2002).

In the **‘screen-and-treat’ programme** (Study I), all individuals aged 15 to 40 years living in Vammala according to the National Population Register received by mail in 1996 a questionnaire and an invitation to give a blood sample for *H. pylori* antibody test. Subjects who had moved to Vammala after updating of the Register were encouraged to participate in the study by announcements in local newspapers. The filled questionnaires were collected at the same visit in Vammala Health Center as the blood samples were taken. A total of 3092 (74%) of the 4174 subjects invited by mail and further 234 subjects invited by newspapers gave a blood sample (Figure 2). Of the 3326 participants, 1758 (52.9%) were females. In 1997-2000, the programme was extended to reach each year all the 15 and 45-year-old inhabitants of Vammala, who were invited by a letter to participate (Figure 2). A total of 717 teenagers including 369 (51.5%) females, (92.5% of those 775 invited), and a total of 584 adults including 318 (54.4%) females, (63% of those 927 invited), gave a blood sample.

In Study II, the data from 561 consecutive adult outpatients (age range, 16 to 91; median age 56 years, 339 (60.4%) female), who had undergone gastroscopy due to clinical indications at the primary care in Vammala Health Centre, Vammala, Finland, from December 1998 to November 2002, was analysed. Patients who had been successfully treated for helicobacter infection earlier were excluded.

Serum samples collected from participants belonging to the original Group A and Group B in 1973 and 1994 and stored at -20C° were reanalysed in Studies III and IV (Figure 1).

In Study III, we reanalyzed data on paired sera stored at -20C° from a total of 560 subjects. The paired sera were obtained from 224 persons [initial age-range in 1973, 15-59 years; median age, 33 years; 131 (58.5%) females] ((from the original Group

A) who had taken part in the population based health survey in Vammala, Finland in 1973 and who had given a follow-up blood sample in 1994 in the follow-up study on the seroprevalence of *H. pylori* infection (Series B), and from 336 persons [initial age-range 30 to 58; median age 44 years, 207 (61.6%) females] who had participated both in another health survey (Mini-Finland Health Survey, see below) during 1978-1980 and in a follow-up study on asthma and atopic diseases during 1997-1998 (SeriesA). The Social Insurance Institution of Finland performed a comprehensive population-based health survey (Mini-Finland Health Survey) during 1978-1980 in Finland following pilot studies carried out in 1977 (Aromaa et al., 1989). A sample of 8000 subjects, representing all Finns aged 30 or over, was studied. The sampling method was a two-stage stratified cluster design and the sample was self-weighting. A total of 7217 people (90% of the sample) participated in a basic health examination including collection of a blood sample. A total of 403 adults, who initially took part in the Mini-Finland Health Survey, participated in a follow-up study on asthma and atopic diseases two decades (range 17.7 to 20.1 years, mean 19.0 years) later during 1997-1998 (Karjalainen et al., 2002). Of these 403 adults, 139 persons either had asthma in the original survey or had developed asthma during the follow-up period, whereas 264 persons not suffering from asthma or chronic obstructive pulmonary disease served as controls. Cases and controls were matched for age, gender and area of residence. Of the 403 adults, paired serum samples, stored at -20°C, from 336 persons [initial age-range 30 to 58; median age 44 years, 207 (61.6%) females], were available for the present Study III.

In Study IV, we analysed the frozen sera collected from altogether 911 subjects in Vammala in 1973 (n=408, from the original Group A) and in 1994 (n=503, from the original Group B). Paired serum samples were available from 221 individuals of those original 224 paired samples obtained in both 1973 and 1994 (from Group A) and were also examined.

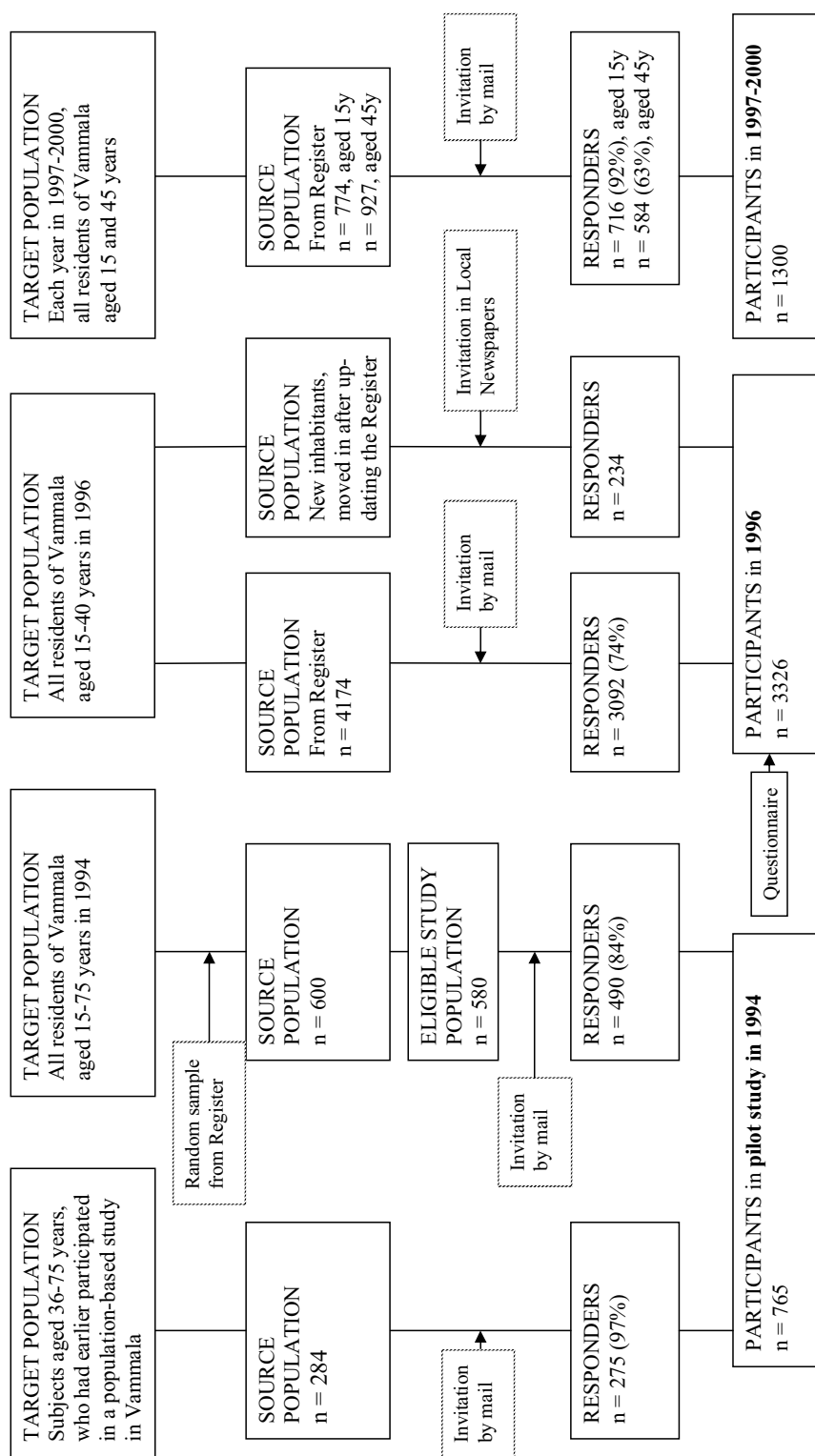


Figure 2. Design of the pilot study in 1994 and the population-based 'screen-and-treat' *H. pylori* programme in Vammala, Finland, in 1996-2000. Register, National Population Register; y, years.

ETHICAL CONSIDERATIONS

The Ethics Committee of the Hospital District of Pirkanmaa approved the study and the participants gave their written informed consent. The studies were conducted according to the Declaration of Helsinki. Most of the frozen sera utilized in Studies III and IV had been collected before present legislation which provides a written consent from the study subjects. Public Health Institute-wide legislation authorized the examination of frozen sera obtained from former population-based health studies or clinical studies (following so called ‘principle of silent permission’ of the legislation).

METHODS

Serum tests

H. pylori antibodies

Serum samples were stored at -20C° until analysed. The paired sera were analysed in parallel. Antibodies to *H. pylori* were measured separately for IgG and IgA classes by an in-house EIA, as described earlier and demonstrated (with cut-off titres of 700 for IgG and 70 for IgA) a sensitivity and a specificity of 94% and 93% for IgG, and 73% and 95% for IgA, respectively, in detecting *H. pylori* infection as compared to histology and culture (Kosunen et al., 1997). *H. pylori* antibodies were also measured by commercially available EIAs Pyloriset EIA-G and Pyloriset EIA-A (Orion Diagnostica, Espoo, Finland) with a sensitivity and a specificity of 99% and 94% for the IgG test and 95% and 92% for the IgA test, respectively, with cut-off titres of ≥ 300 for IgG and ≥ 250 for IgA (Oksanen et al., 1998). In 2000 the manufacturer replaced the commercial test with new versions of the test, Pyloriset EIA-GIII and Pyloriset EIA-AIII (Orion Diagnostica, Espoo, Finland). Local validation of the new versions of the test resulted in a sensitivity and a specificity of 100% and 99% for the IgG test and 52% and 97% for the IgA test, respectively, when used in the 15 to 49-

year-old subjects (Study II) and when values of 30 or higher were considered positive instead of the cut-off value of 20 recommended by the manufacturer.

The in-house EIA alone was used in the pilot study as part of Study I, in Study III in Series B, and in Study IV. Pyloriset EIA-III alone was used in Study III in Series A. Both the in-house EIA and Pyloriset EIA were used in Study I in the population-based 'screen-and-treat' programme. The former version of the Pyloriset EIA was in use in Study I until we started to use the new version of the test in 2000. In Study II, serum samples were analysed by using both in-house EIAs and Pyloriset EIA-III test kits, but only the results of Pyloriset EIAs were published.

Pepsinogen I

The sera were stored at -20C° until analysed. The serum PGI concentration was measured with an immunoenzymometric commercial assay (Gastroset PG1, Orion Diagnostica, Espoo, Finland). According to manufacturer's instructions, PG1 values below 28 microg/liter were considered low. With this limit, the sensitivity and specificity for Gastroset PG1 were 81% and 99%, respectively (Oksanen et al., 2001). PGI concentrations were measured in Studies I and II, but PGI results were reported only in Study II.

CagA antibodies

The sera were stored at -20C° until analysed. Antibodies to the *H. pylori* CagA antigen were determined using a recombinant CagA fragment by EIA. An optical density ratio (ODR) of ≥ 0.35 was considered positive. The sensitivity and specificity of the test were 94.4% and 92.5%, respectively, in a US population (Blaser et al., 1995). The assays were performed at least in duplicate. CagA antibodies were assayed in Study IV.

Gastroscopy and biopsies

Gastrosopies (Study II) were performed in a routine manner by A.S-R. with two biopsies from the gastric antrum, two biopsies from the gastric corpus for histologic

examination. Additional biopsies, one from the antrum and one from the corpus, were obtained for culture. The evident macroscopical pathology was recorded.

***H. pylori* culture**

The gastric biopsies (Study II) were mailed for culture in Transpocult-tubes (Orion Diagnostica, Espoo, Finland). The minced specimens were inoculated for *H. pylori* for up to 12 days on Brucella agar plates (Becton, Dickinson, Sparks, MD, USA) supplemented with whole horse blood (7%) and on selective Brucella agar plates containing Iso-Vitalex (1%), vancomycin (6mg/L), amphotericin B (2mg/L) and nalidixid acid (20mg/L). The plates were incubated for up to 12 days in spec. atmosphere at 37C°. *H. pylori* was identified by colony appearance, Gram staining, and positive reactions for oxidase, urease, and catalase.

Histology

Formalin fixed-biopsy specimens (Study II) were embedded in paraffin and tissue sections were cut at three levels per biopsy and placed on one slide. The sections were stained with haematoxylin and eosin, Alcian blue (pH 2.5) / PAS stain, and modified Giemsa stain. Gastritis was classified according to the Sydney System (Price, 1991) in a blinded arrangement by one pathologist (J.M.).

Urea breath test

UBTs (Study I) were performed after an overnight or at least four hours fast. An aqueous solution of 150 ml water containing 75 mg citric acid solution was given first and a breath sample was collected 5 min after ingestion, and an additional 50 ml of water containing ¹³C-labelled urea was given. A new breath sample was obtained 30 min after the second ingestion. The samples were sent by mail to a private laboratory (Keskuskadun laboratorio, Helsinki, Finland) for analysis by gas chromatography. The cut-off values were determined as delta over baseline (DOB) >4 ‰ positive, <2‰ negative, and 2-4‰ uncertain. Subjects were advised not to take acid suppressive medication in the preceding 7 days and antimicrobials in the preceding month before the UBT.

Diagnosis of *H. pylori* infection

In Study I in the pilot study, the diagnosis of *H. pylori* infection was based on the IgG antibody result (in-house EIA), and in the case of a negative IgG result on the positive IgA antibody result. In the population-based 'screen-and-treat' programme (Study I), all sera were tested with both in-house EIA and Pyloriset EIAs and diagnosis of *H. pylori* infection was based on a positive IgG antibody result in most of the subjects. In the case of discrepancy between the IgG test results, the *H. pylori* positivity was based on that IgG test demonstrating a result above the cut-off value. In the case of negative results in both the IgG tests, the subjects were regarded as *H. pylori* positive if the in-house IgA antibody test was positive.

In Study III, persisting *H. pylori* infection was defined as a positive IgG antibody result at the beginning and at the end of the follow-up period. When evaluating the changes in IgA and IgG antibody levels and antibody status, the positivity was based on antibody level exceeding the cut-off value for the test evaluated. Seroconversion and seroreversion was defined on the basis of IgG antibody result.

In Study IV, the *H. pylori*-positivity was defined as a positive result in any of the three serological assays used (in-house IgG, in-house IgA and CagA). *H. pylori*-negative status was defined when all three assays were negative. In those subjects with paired sera from 1973 and 1994, seroconversion and seroreversion were defined on the basis of IgG response to the acid glycine extracted *H. pylori* antigen (in-house IgG).

Patients in Study II were considered *H. pylori* positive if culture or histology were positive and *H. pylori* negative if both culture and histology were negative.

UBT was used in border-line antibody result cases at baseline to confirm the diagnosis of *H. pylori* infection (Study I).

Eradication therapy

All *H. pylori* antibody-positive subjects were offered antimicrobial therapy in the pilot study in 1995 and in the population-based 'screen-and-treat' programme started in 1996 (Study I) as a part of a general practice consultation by A. S-R., and all treated subjects were invited by mail to a new general practice consultation and a serologic follow-up six months later.

In the pilot study, the first-line therapy included amoxicillin (500mg) q.i.d., metronidazole (400mg) t.i.d. and lansoprazole (30mg) b.i.d. for 14 days, and the second-line therapy (for those allergic to amoxicillin and for those with a treatment failure in triple therapy) consisted of tetracycline (500mg) q.i.d., metronidazole (400mg) t.i.d., and bismuth subcitrate (240mg) and lansoprazole (30mg) b.i.d. for 14 days.

In the population-based study, the only exclusion criterion was alcoholism due to the possibility of an antabus effect of metronidazole. In the case of pregnancy, the eradication therapy was offered after lactation. The first-line therapy included amoxicillin (1000mg) b.i.d., metronidazole (400mg) t.i.d. and lansoprazole (30mg) b.i.d. for 7 days. Subjects allergic to amoxicillin were given tetracycline (500mg) q.i.d., metronidazole (400mg) t.i.d., bismuth subcitrate (240mg) and lansoprazole (30mg) b.i.d. for 7 days. After treatment failure the second-line treatment was amoxicillin (1000mg), clarithromycin (500mg) and lansoprazole (30mg) b.i.d. for 7 days, or metronidazole (400mg) t.i.d., and clarithromycin (500mg) and lansoprazole (30mg) b.i.d. for 7 days for those allergic to amoxicillin. The patients could purchase the medicines from local pharmacies for a reduced price, due to support from pharmaceutical companies.

Criterion for cure

Pre- and post-treatment sera (Study I) were always tested in the same run to determine the decline of antibody levels. The criterion for successful eradication therapy was a decline of at least 40% in the IgG antibody level in six months.

UBT (Study I) was used in border-line cases at follow-up, and to confirm eradication failures before retreatment in the population-based 'screen-and-treat' programme.

Questionnaire

A printed questionnaire (Study I) was used in 1996 to obtain statistically useful or personal information from individuals. The 65 questions formulated for this study covered the background of the participant, information on childhood household, smoking habits, consumption of alcohol, use of acetylsalicylic acid (ASA) and NSAIDs in the previous six months, diagnosed *H. pylori*-associated diseases, gastrointestinal symptoms evaluated by frequency and intensity (5-graded scale) and doctor consultations, medical examinations, medications and sick leave due to upper abdominal symptoms in the previous six months. Quality of life and situation in life were also documented.

Statistical analysis

The statistical significance of the differences between *H. pylori* antibody-positive and antibody-negative subjects in Study I was tested first using Pearson's chi-square, Mann-Whitney's U, and Fisher's exact tests, and the confounding factors were controlled by Mantel-Haenszel's test and by multivariate regression analysis. Each risk factor remaining statistically significant was analysed separately in a univariate model, and those factors remaining statistically significant were further analysed using a multivariate logistic regression model. The results were expressed as odds ratios (OR), 95% confidence intervals (CI) and p-values. Two-sided p-values less than 0.05 were considered statistically significant.

The sensitivity, specificity, positive and negative predictive values were determined for each EIA test and compared with the prevalence of *H. pylori* as based on histology or culture or both in Study II. The statistical significance of the improvement of the specificity figures was evaluated using Pearson's Chi-Square and Fisher's Exact tests, and a trend in proportion by age was examined using a linear by linear test.

The statistical significance of the increase in IgA titres in Study III was evaluated by using Wilcoxon's signed rank pair test, and the increase in the number of IgA responders was examined by using McNemar's test. Differences in frequencies of response to Cag antigen among groups in Study IV was studied by Pearson's chi-square test, and the magnitude of the responses in different groups was examined using the Student's t test for independent samples.

The statistical analysis were done by using SPSS 12.0 software packages (SPSS Inc, Chicago, IL, USA). In Study II, StatXact 5.0 (Cytel Inc, MA, USA) was also used.

RESULTS

The 'screen-and-treat' study (Study I)

Participation rates

The participation rates in the 'screen-and-treat' programme in Vammala, Finland are summarised in Table 1 (Study I). A total of 3316 (99.7%) of the 3326 participants of the population-based programme (Study I) returned the questionnaire, including all of the 406 antibody-positive subjects and 2910 (99.7%) of the 2920 antibody-negative subjects.

Table 1. Participation rates in different phases of the 'screen-and-treat' programme in Vammala, Finland. NPR, National Population Register.

YEAR, source	AGE	Invited	Participated		Female	
		N	N	%	N	%
1994, pilot study	15-75	864	765	88.5	411	53.7
1996, from NPR	15-40	4174	3092	74.1		
1996, newspaper invitation	15-40		234			
1996, total	15-40		3326		1758	52.9
1997-2000, from NPR	15	774	716	92.4	369	51.5
1997-2000, from NPR	45	927	584	63.0	318	54.4

Risk factors for non-compliance

We evaluated the reasons for non-compliance by analysing the questionnaire results. Of the 406 antibody-positive participants, 46 (11.3%) did not show up to receive eradication therapy. Current smoking was the only characteristic associated with the not appearing at the consultation, as 64% of those not showing up and 34% of those consulting the general practitioner to receive eradication therapy were current smokers ($p < 0.0001$, OR 3.3, 95% CI 1.7 to 6.4). Among those consulting the general practitioner, the parents' education was found to be associated with the non-compliance so that, a higher basic education of both mother and father was more common among participants who were drop-outs than among those who completed the follow-up (Table 2). In addition, current smoking and current alcohol consumption were more common in those who had dropped out from the follow-up (Table 2).

Table 2. Odds ratios with 95% confidence intervals and prevalences of risk factors for non-compliance in the follow-up after treatment. Results from univariate analyses*. Information collected by a questionnaire filled out by the participant at the beginning of the 'screen-and-treat' programme. Ref.category, reference category.

	Drop outs		Responders		P-value	OR	95% CI	
	n	%	n	%			Lower	Upper
Mother's basic education					0,006			
elementary school (ref.category)	23	64,9	244	83,0				
middle school or comprehensive school	8	21,6	42	14,3	0,002	6,35	1,93	20,96
upper secondary school	5	13,5	8	2,7	0,084	3,28	0,851	12,65
Father's basic education					0,037			
elementary school (ref.category)	28	73,7	253	87,5				
middle school or comprehensive school	6	15,8	28	9,7	0,019	4,52	1,28	15,96
upper secondary school	4	10,5	8	2,8	0,265	2,33	0,526	10,35
Smoking								
current smoking**	25	62,5	91	30,5	<0,0001	3,79	1,91	7,52
Alcohol consumption								
current alcohol consumption**	36	90,0	222	74,7	0,041	3,04	1,05	8,85
former consumption in those who have stopped	10	90,9	42	42,9	0,015	13,3	1,64	111,1

*For binary logistic regression analyses, the variables measuring educational level were entered as categorical variables, and other variables as dichotomical variables. SPSS 12.0.1. software packages (SPSS, Inc., Chicago, Ill.) was used.

** The question was related to the last six months.

Prevalence rates of *H. pylori* infection

The seroprevalence rates increased by age (Study I) in 1994 from 4.6% to 69.4% in 15 to 75-year-old subjects (Table 3) and in 1996 from 4.2% to 22.2% in 15 to 40-year-old individuals in the 5-year age groups (Table 4). Each year in 1997-2000 all 15 and 45-year-old persons were invited to the programme and the seroprevalence rate was 3.2% among the teenagers and 27.4% among the 45-year-old adults (Table 5).

The initial *H. pylori* seroprevalence rates were calculated to have decreased from 39% to 19% in the pilot study, from 12% to 4% among subjects aged 15 to 40 years, from 3% to 2% among the 15-year-old subjects, and from 27% to 12% among the 45-year-old subjects (Tables 3-5).

Table 3. Seroprevalence rates in males, in females, and in both genders combined at base-line in 1994, and calculated seroprevalence rates after the 'screen-and-treat' programme in 5-year age-groups, in the pilot study in Vammala, Finland. AB+, number of antibody-positive* subjects; %calc, calculated seroprevalence rate.

AGE	M a l e		F e m a l e		A l l participants			A f t e r treatment	
	AB+	%	AB+	%	ALL	AB+	%	AB+	%calc
15-20	0	0.00	2	8.00	44	2	4.55	1	2.27
21-25	2	14.29	1	5.26	33	3	9.09	1	3.03
26-30	1	7.14	4	19.05	35	5	14.29	1	2.86
31-35	2	8.33	3	13.04	47	5	10.64	2	4.26
36-40	8	21.62	12	27.27	81	20	24.69	6	7.41
41-45	11	29.73	21	41.18	88	32	36.36	15	17.05
46-50	13	30.23	21	42.86	92	34	36.96	18	19.57
51-55	16	48.48	19	44.19	76	35	46.05	16	21.05
56-60	22	55.00	22	55.00	80	44	55.00	22	27.50
61-65	23	62.16	16	53.33	67	39	58.21	14	20.90
66-70	16	64.00	21	60.00	60	37	61.67	21	35.00
71-75	25	80.65	18	58.06	62	43	69.35	31	50.50
total	139	39.27	160	38.93	765	299	39.08	148	19.35

* In-house IgG and IgA antibody results combined.

Table 4. Seroprevalence rates in males, and in females, and in both genders combined at base-line in 1996, and calculated seroprevalence rates after the intervention in 5-year age-groups in the 'screen-and-treat' programme in Vammala, Finland. AB+, number of antibody-positive* subjects; %calc, calculated seroprevalence rate.

AGE	M a l e		F e m a l e		A l l participants			A f t e r treatment	
	AB+	%	AB+	%	ALL	AB+	%	AB+	%calc
15-20	21	4.44	18	4.01	922	39	4.23	14	1.52
21-25	17	8.99	24	8.57	469	41	8.74	8	1.71
26-30	26	10.24	35	11.36	562	61	10.85	23	4.09
31-35	56	17.45	57	15.49	689	113	16.40	35	5.08
36-40	79	23.87	73	20.68	684	152	22.22	41	5.99
total	199	12.69	207	11.77	3326	406	12.21	121	3.64

* IgG and IgA antibody results combined, assayed by using in-house and Pyloriset EIAs.

Table 5. Seroprevalence rates in both genders separately and combined at base-line in 1997-2000, and calculated seroprevalence rates after the programme in 15 and 45-year-old participants in the ‘screen-and-treat’ programme in Vammala, Finland. AB+, number of antibody-positive* subjects; %calc, calculated seroprevalence rates; yr, year.

AGE	YEAR	M a l e		F e m a l e		A l l participants			A f t e r treatment	
		AB+	%	AB+	%	ALL	AB+	%	AB+	% calc
15 yr	1997	3	3.33	2	2.00	190	5	2.63	1	0.53
	1998	2	2.22	3	2.91	193	5	2.59	4	2.07
	1999	3	3.30	3	3.49	177	6	3.39	5	2.82
	2000	1	1.32	6	7.50	156	7	4.49	5	3.21
	97-00	9	2.59	14	3.79	716	23	3.21	15	2.09
45 yr	1997	24	31.17	27	30.68	165	51	30.91	18	10.91
	1998	22	28.20	24	26.67	168	46	27.38	18	10.71
	1999	14	24.14	17	24.29	128	31	24.22	16	12.50
	2000	14	26.41	18	25.71	123	32	26.02	19	15.45
	97-00	74	27.82	86	27.04	584	160	27.40	71	12.16

* IgG and IgA antibody results combined, assayed by using in-house and Pyloriset EIAs.

Eradication rates

All *H. pylori*-antibody-positive individuals were offered antimicrobial therapy (Study I). In the pilot study 157 (52.5%) of those infected were treated and the eradication rate was 89.8%. The eradication rates after first treatment were 82.2% and 77.6% per-protocol and 71.9% and 62.9% in intention-to-treat in subjects invited to the ‘screen-and-treat’ programme in 1996 and 1997-2000, respectively. After the second treatment, the per-protocol eradication rates were 83% and 83%, and the-intention-to-treat eradication rates were 66% and 62% in subjects invited to the programme in 1996 and in 45-year-old subjects invited in 1997-2000, respectively. The two teenagers who were treatment failures in 1997-2000 refused a second course of treatment.

Table 6. Grade and location of gastric atrophy in *H. pylori*-positive* and -negative** subjects and prevalence of low Pepsinogen I values in patients with corpus atrophy. Number of patients with the indicated results. PGI, pepsinogen I.

Grade of atrophy ^a	<i>H. pylori</i> +		<i>H. pylori</i> -	
	PGI <28 ^b / corpus atrophy (prevalence)	antrum atrophy	PGI <28 / corpus atrophy (prevalence)	antrum atrophy
0	1 / 130	154	10 / 328	366
1	1 / 31 (3.4%)	17	4 / 13 (31%)	7
2	4 / 14 (29%)	5	10 / 16 (62%)	3
3	1 / 4 (25%)	2	21 / 22 (95%)	0
unknown ^c	0 / 2	3	0 / 1	4
total	7 / 181	181	41 / 380	380

* *H. pylori*+, *H. pylori* positivity was based on positive histology or culture or both.

** *H. pylori* -, both histology and culture were negative.

^a Grade of corpus/antrum atrophy according to the Sydney System

^b Pepsinogen I (Gastroset PGI, Orion Diagnostica, Espoo, Finland), low value <28microg/liter.

^c There were 3 patients with antrectomy and 4 patients with superficial biopsy specimens without glands.

Accuracy of *H. pylori* antibody assays (Study II), with special emphasis on atrophic gastritis

Presence of *H. pylori* and atrophic gastritis on gastric mucosa, and endoscopic findings

Of the 181 *H. pylori*-positive patients, culture was positive in 173 (95.6%), and microscopy of the histological preparations was positive in 161 (88.9%) ($p = 0.0305$). Atrophic gastritis was detected in histology in 66 (36.5%) of the 181 patients with *H. pylori* gastritis, and in 54 (14.2%) of the 380 *H. pylori*-negative patients ($p < 0.0001$). The grade and location of gastric atrophy among *H. pylori*-positive and -negative subjects, and the prevalence of low PGI values in patients with corpus atrophy is shown in Table 6. Atrophic gastritis based on histology or a low PGI

Table 7. Characteristics of the patients with a false positive antibody result in any of the four tests (combination of IgG and IgA results) but *H. pylori*-negative results in culture and histology. N, normal gastric mucosa in histology; G, chronic gastritis without helicobacters in biopsy specimens; CA, gastric malignancy.

Age-group (n)	Number (%) of false positives	Number of histological findings										CA
		N	G	grade of corpus-atrophy ^a (PGI < 28 microg/liter ^b)				grade of antrum-atrophy ^a				
				0	1	2	3	0	1	2	3	
15-49 (157)	6 (3.8%)	5	1	6 (0)	-	-	-	0	-	-	-	0
50-64 (192)	17 (8.8%)	9	8	11 (0)	-	2 (0)	4 (0)	17	-	-	-	0
≥ 65 (212)	43 (20.3%)	4	39	23 (3)	8 (3)	7 (5)	4 (4) ^c	35	6	1 ^d	-	3 ^e

^a Grade of corpus/antrum atrophy according to the Sydney System (range 0-3).

^b Pepsinogen I (Gastroset PGI, Orion Diagnostica, Espoo, Finland).

^c 1 patient with superficial biopsy specimen without glands, explaining missing data in corpus atrophy numbers.

^d 1 patient with antrectomy, explaining missing data in antrum atrophy numbers.

^e 2 adenocarcinomas and 1 lymphoma.

value was more common in the older age groups, appearing in 10.2%, 17.2%, and 39.6% of the patients aged 15-49, 50-64, and >65 years, respectively ($p = 0.0001$).

Characteristics of the patients with a false positive antibody result but *H. pylori*-negative results in culture and histology, related to gastric atrophy, are presented in Table 7. Peptic ulcer disease was detected in 22 (12.1%) infected patients and in five (1.2%) *H. pylori*-negative patients ($p < 0.0001$).

Accuracy of *H. pylori* antibody assays

The accuracy of *H. pylori* antibody assays was evaluated in the age-groups of 15-49, 50-64, and ≥65 years. The sensitivities, specificities, PPVs, and NPVs of both Pyloriset and in-house assays are shown in Table 8, although only the results of Pyloriset EIA were published in article (Study II). The sensitivities of the both IgG tests were high in all age-groups, but the specificities for the in-house and Pyloriset IgG tests declined by age-group from 99.3 and 98.5% for those aged 15 to 49 years to

Table 8. Sensitivities, specificities, PPVs, and NPVs of *H. pylori* EIA antibody tests for different age-groups for all patients and after exclusion of patients with atrophic gastritis ^a. PPV, positive predictive value; NPV, negative predictive value.

Age-group (years) prevalence (%) ^b	No. of patients	Test	Sensitivity	Specificity	PPV	NPV	p-value for increase in specificity
15 - 49	157						
<i>H. pylori</i> + 13.4%		Pyloriset EIA-GIII	100 (100)	99.3 (99.3)	95.4 (95.4)	100 (100)	
Histology+ 12.1%		In-house IgG	100 (100)	98.5 (98.5)	91.3 (91.3)	100 (100)	
Culture+ 13.4%		Pyloriset EIA-AIII	52.4 (52.4)	97.1 (97.1)	73.3 (73.3)	92.9 (92.9)	
		In-house IgA	57.1 (57.1)	100 (100)	100 (100)	93.8 (93.8)	
		Pyloriset EIA-GIII + AIII ^c	100 (100)	96.3 (96.3)	80.8 (80.8)	100 (100)	
		In-house IgG + IgA	100 (100)	98.5 (98.5)	91.3 (91.3)	100 (100)	
50 - 64	192						
<i>H. pylori</i> + 37.5%		Pyloriset EIA-GIII	98.6 (98.6)	94.2 (97.4)	91.0 (95.9)	99.1 (99.1)	ns ^d
Histology+ 33.3%		In-house IgG	100 (100)	90.8 (94.8)	94.0 (92.3)	100 (100)	ns
Culture+ 36.5%		Pyloriset EIA-AIII	80.6 (80.6)	91.7 (94.8)	85.3 (90.6)	88.7 (88.7)	ns
		In-house IgA	75.0 (75.0)	96.7 (98.3)	93.1 (96.4)	86.6 (86.6)	ns
		Pyloriset EIA-GIII + AIII	98.6 (98.6)	89.2 (93.0)	84.5 (89.9)	99.1 (99.1)	ns
		In-house IgG + IgA	100 (100)	90.0 (93.9)	85.7 (91.1)	100 (100)	ns
≥ 65	212						
<i>H. pylori</i> + 41.5%		Pyloriset EIA-GIII	98.9 (98.9)	75.0 (93.0)	73.7 (92.5)	98.9 (98.9)	<0.0001
Histology+ 36.8%		In-house IgG	98.9 (98.9)	73.4 (88.3)	72.5 (87.9)	98.9 (98.9)	0.0049
Culture+ 38.7%		Pyloriset EIA-AIII	86.4 (86.4)	79.0 (92.4)	74.5 (90.5)	89.1 (89.1)	0.0043
		In-house IgA	68.2 (68.2)	82.3 (93.6)	73.2 (89.5)	78.5 (78.5)	0.009
		Pyloriset EIA-GIII + AIII	100 (100)	70.2 (86.1)	70.4 (86.3)	100 (100)	0.0044
		In-house IgG + IgA	100 (100)	72.6 (87.4)	72.1 (87.1)	100 (100)	0.0062
All	561						
<i>H. pylori</i> + 32.3%		Pyloriset EIA-GIII	98.9 (98.9)	89.7 (96.9)	82.1 (82.1)	99.4 (99.4)	<0.0001
Histology+ 28.7%		In-house IgG	99.4 (99.4)	87.9 (94.3)	79.6 (90.0)	99.7 (99.7)	0.0023
Culture+ 30.8%		Pyloriset EIA-AIII	80.1 (80.1)	89.5 (95.0)	78.4 (88.9)	90.4 (90.4)	0.0055
		In-house IgA	69.6 (69.6)	93.2 (97.5)	82.9 (93.3)	86.5 (86.5)	0.005
		Pyloriset EIA-GIII + AIII	99.4	85.5(nc) ^e	76.6 (nc)	99.7 (nc)	nc
		In-house IgG + IgA	100	87.4 (nc)	79.0 (nc)	100 (nc)	nc

^a Atrophic gastritis was verified by histology or a Pepsinogen I value of less than 28 microg/liter. Results after exclusion of those patients with atrophic gastritis and a false positive antibody result are shown in parentheses.

^b *H. pylori*+, prevalence of *H. pylori* as based on histology or culture or both; Histology+, prevalence of *H. pylori* as based on histology alone; Culture+, prevalence of *H. pylori* as based on culture alone.

^c Combination of IgG and IgA results.

^d Non significant.

^e Not calculated because of different numbers of false positive subjects in IgG and IgA tests.

75.0 and 73.4% for those aged ≥ 65 years, respectively. The sensitivities of the both IgA tests were low in the youngest age-group, but increased by age-group.

The exclusion of false positive results for patients with atrophic gastritis as based on histology or a PGI value < 28 microg/liter improved the specificities for the in-house and Pyloriset IgG tests to 97% and to 93%, and to 95% and to 94%, in patients aged 50 to 64 years and 65 years or more, respectively.

Changes in *H. pylori* antibody levels (Study III)

In series A in Study III, seven (2.1% of all 336 subjects) originally antibody negative subjects in the Mini-Finland Study had during the two decades' follow-up developed a positive IgG response and five of them had also become positive for IgA antibodies. The calculated annual rate of new infections was 0.1%. The number of IgG seroreverters was 35 (10.4% of 336) resulting in a calculated annual rate of 0.5% for seroreversions. Of these, there was a change in IgA status from positive to negative in 15 subjects, whilst in 9 subjects, despite negative IgG serology, there was persisting IgA positivity at follow up.

Within the follow-up period of two decades among the 268 subjects (from both Series A and Series B) with a persisting positive IgG response, there was an increase in the number of IgA seroconverters, which did not reach statistical significance (Table 9). In the 197 subjects with a persisting positive IgA and IgG response during the follow-up period, the median IgA titres increased by 48% ($p < 0.0001$) and 22% ($p = 0.0241$) in Series A and B, respectively (Table 10). The changes in the median IgG titres (-1% and -18% in Series A and Series B, respectively) were not significant.

Table 9. IgA antibody status during *H. pylori* infection in 268 subjects with a persisting positive IgG response.

Initial	Follow-up	Number	Proportion (%)	p-value for change
Negative	Negative	39	14.5	0.1102*
Positive	Positive	197	73.5	
Negative	Positive	21	7.8	
Positive	Negative	11	4.1	

* The increase in the number of IgA responders was evaluated using McNemar's test.

Table 10. Long-term changes in median IgG and IgA titres during *H. pylori* infection in 197 subjects with a persisting positive IgG and IgA response.

	Vammala study n = 57		Mini-Finland study n = 140	
	Median IgG titre	Median IgA titre	Median IgG titre	Median IgA titre
Initial	5500	180	2940	885
Follow-up	4500	220	2910	1310
Change of medians (%)	- 18.2%	+ 22.2%	- 1.0%	+ 48.0%
p-value for change*	0.5000	0.0241	0.0932	< 0.0001
EIA-test used	in-house IgG	in-house IgA	Pyloriset EIA-GIII	Pyloriset EIA-AIII

* Statistical significance for the change in medians was examined with Wilcoxon's signed rank pair test.

Table 11. Change in seroprevalence (%) of *H. pylori* antibodies against acid glycine extract (in-house EIA) and CagA antigen between 1973 and 1994 by age. *H.pylori*+, positive *H. pylori* antibodies of classes IgG and IgA or both; CagA+, positive CagA antibodies; ns, non significant. 1973 n= 408, 1994 n=503.

Age	<i>H. pylori</i> + CagA+			<i>H-pylori</i> + CagA -		
	1973	1994	P-value	1973	1994	P-value
14-44 years	34.3	8.0	<0.0001	11.6	5.9	0.03
≥ 45 years	40.7	31.6	ns	30.7	24.1	ns

CagA-antibodies (Study IV)

Validation of CagA threshold in the tested population

As gastric biopsies were not obtained from the subjects in Study IV, we used sera from 65 subjects who were positive both in 1973 and in 1994 for both IgG and CagA antibodies as a gold standard to define subjects being persistently infected with CagA-positive *H. pylori* strains. For these 130 sera, the mean (SD) ODR in the CagA assay was 0.78 (0.22). By subtracting two SD-intervals from the mean ODR, we defined a level that was 97.5% likely to represent truly positive CagA status. This value (0.34) was almost identical to the prior cut-off value of 0.35 determined previously (Blaser et al., 1995).

Changes in the seroprevalence rate of CagA antibodies

The seroprevalence of CagA antibodies declined from 36.5% in 1973 to 20.4% in 1994 in Vammala. The decline was largest among younger subjects aged 14 to 44 years (Table 11). We found 14 (3.4%) subjects in 1973 and 20 (3.9%) subjects in 1994, who were CagA antibody-positive but demonstrated negative result for in-house IgG and IgA antibodies.

Table 12. Stability of antibody responses to *H. pylori* antigens in 21 years follow-up in 95 subjects who were *H. pylori* IgG antibody-positive in 1973.

Assay	Number of subjects positive with indicated assay			Antibody level	
	1973	1994	%	1973	1994
<i>H. pylori</i> IgG*	95	87	91.6	3179	3009
<i>H. pylori</i> IgA*	59	54	91.5	180	201
CagA IgG**	69	69	100	0.75 (0.24)	0.80 (0.21)

* In-house IgG or IgA, shown as reciprocal geometric mean titers (RGMT)

** CagA IgG, shown as mean optical density ratio (ODR) units (SD)

Changes in overall *H. pylori* positivity and CagA antibody status

Paired sera from 221 subjects obtained both in 1973 and 1994, were examined to study whether *H. pylori* or CagA serostatus had changed. In total, only 12 (5.4%) subjects had a change in overall *H. pylori* status during the two decades follow-up period. In nine subjects (7.8% of the subjects who were seronegative in 1973), the overall status changed from negative to positive (giving a crude seroconversion rate of 0.4% per year), and eight of the nine also became positive in the CagA assay. In three subjects (2.8% of the 106 subjects who were seropositive in 1973), the overall status changed from positive to negative (the crude seroreversion rate was 0.13% per year), and all the three had been CagA antibody-negative in 1973 and remained negative in 1994.

Changes in the levels of CagA antibodies

The paired sera from 221 subjects obtained both in 1973 and 1994, were also examined to determine the degree of fluctuation in the level of antibodies present among *H. pylori*-positive subjects in the two decades follow-up period. In Table 12 is shown the stability of antibody responses to *H. pylori* antigens (measured by the in-house EIAs for IgG and IgA antibodies, and the EIA for CagA IgG antibodies). Among those who were CagA-positive in 1973 and 1994, the calculated mean annual increase in antibody level was 0.3%.

Risk factors for *H. pylori* infection (Study I)

The printed questionnaire including 65 questions was used to obtain statistically useful data on possible risk factors for *H. pylori* infection from individuals who participated in the ‘screen-and-treat’ study. The questionnaire was returned by 99.7% of the participants in 1996. Statistically significant risk factors for *H. pylori* infection and characteristics of *H. pylori*-positive subjects compared to *H. pylori*-negative ones are shown in Table 13.

Low education

Parents’ education was associated with *H. pylori* infection so that, in the case of a mother both low basic and low vocational education and in the case of a father low basic education reached statistical significance. Participant’s basic education was associated with the infection, as well.

Childhood household

In childhood household, the crowding variables (number of family members, number of persons sharing a bedroom, sharing the bed with some one else) and using well water were associated with *H. pylori* infection (Table 13), but kissing habits between the participant and the parents and the siblings were not associated with the infection.

Life style

Current smoking and current alcohol consumption were significantly more common among *H. pylori*-infected than *H. pylori*-negative individuals as shown in Table 14. A higher number of daily cigarettes was found to be associated with *H. pylori* infection, (OR 1.88, 95% CI 1.30-2.71), as 39% (n = 57) and 25.4% (n = 204) of the antibody-positive and -negative smokers, respectively, smoked more than 14 cigarettes daily (p = 0.001). In addition, alcohol consumption was more severe in *H. pylori*-positive subjects (Table 14).

Table 13a. Odds ratios with 95% confidence intervals and prevalences of risk factors in *H pylori*-positive and -negative subjects aged 15 to 40 years. Results from univariate analyses*. Information collected by an epidemiologic questionnaire filled out by the participant at the beginning of the 'screen-and-treat' programme in Vammala, Finland. Ref.category, reference category.

	<i>H pylori</i> positive		<i>H pylori</i> negative		Results from univariate analyses		
	n	%	n	%	P-value	OR	95% CI
Mother's basic education					<0.0001		
elementary school	299	80.6	1690	62.8	<0.0001	4.04	2.29 - 7.14
middle school or comprehensive school	59	15.9	705	26.2	0.039	1.91	1.03 - 3.54
upper secondary school (ref.category)	13	3.5	297	11.0			
Father's basic education					<0.0001		
elementary school	316	85.9	1954	73.5	0.002	2.46	1.39 - 4.37
middle school or comprehensive school	39	10.6	505	19.0	0.624	1.18	0.61 - 2.25
upper secondary school (ref.category)	13	3.5	198	7.5			
Mother's vocational education					<0.0001		
no vocational training	181	51.1	927	35.5	0.002	3.51	1.61 - 7.65
vocational courses	79	22.3	577	22.1	0.026	2.46	1.11 - 5.47
vocational school	63	17.8	586	22.5	0.108	1.93	0.87 - 4.32
college level degree	24	6.8	394	15.1	0.835	1.1	0.46 - 2.61
academic degree (ref.category)	7	2.0	126	4.8			
Participant's basic education					<0.0001		
elementary school	42	11.0	137	5.1	<0.0001	2.86	1.90 - 4.31
middle school or comprehensive school	252	66.0	1740	64.5	0.021	1.35	1.04 - 1.75
upper secondary school (ref.category)	88	23.0	821	30.4			
Childhood home							
having well water	259	68.2	1531	55.8	<0.0001	1.69	1.35 - 2.13
sharing bed with someone	112	29.5	489	17.9	<0.0001	1.92	1.51 - 2.44
no. of family members					<0.0001	1.15	1.09 - 1.21
no. of persons in bedroom					<0.0001	1.3	1.20 - 1.41
Smoking							
current smoking**	144	37.7	738	26.8	<0.0001	1.65	1.32 - 2.06
Alcohol consumption							
current alcohol consumption**	292	76.8	1755	63.8	<0.0001	1.88	1.47 - 2.42
Anfavourable situation¹ of life related to...							
economical situation ¹	360	95.5	2380	87.7	<0.0001	2.97	1.80 - 4.90
housing conditions ¹	270	71.2	1627	59.6	<0.0001	1.68	1.33 - 2.13
overall situation ¹	307	81.0	1980	72.6	0.001	1.61	1.23 - 2.11
¹ very good or good / average, bad or very bad							
Occurence of GORD-symptoms**	189	55.9	1002	42.0	<0.0001	1.75	1.39 - 2.20
Medical examinations and sick leaves because of dyspeptic symptoms							
Gastroscopy	44	11.6	170	6.2	<0.0001	1.99	1.40 - 2.83
X-ray of ventricle	51	13.5	220	8.0	<0.0001	1.79	1.29 - 2.47
being on sick leave**	11	2.9	34	1.2	0.012	2.4	1.21 - 4.79
<i>H. pylori</i> associated diseases							
gastritis	28	7.3	76	2.8	<0.0001	2.79	1.79 - 4.37
peptic ulcer disease	16	4.2	22	0.8	<0.0001	5.45	2.83 - 10.47

*For binary logistic regression analyses, the variables measuring educational level were entered as categorical variables, the crowding variables were entered as continuous variables and other variables as dichotomical variables. SPSS 12.0 software packages (SPSS, Inc., Chicago, Ill.) was used.

** The question was related to the last six months.

Table 13b. Odds ratios with 95% confidence intervals and prevalences of risk factors in *H. pylori*-positive and -negative subjects aged 15 to 40 years. Results from multivariate analysis*. Information collected by an epidemiologic questionnaire filled out by the participant at the beginning of the 'screen-and-treat' programme in Vammala, Finland. Ref.category, reference category.

	<i>H. pylori</i> positive		<i>H. pylori</i> negative		Results from multivariate analysis		
	n	%	n	%	P-value	OR	95% CI
Mother's basic education					<0.0001		
elementary school	299	80.6	1690	62.8	0.001	3.02	1.56 - 5.87
middle school or comprehensive school	59	15.9	705	26.2	0.131	1.73	0.849 - 3.51
upper secondary school (ref.category)	13	3.5	297	11.0			
Father's basic education					ns		
elementary school	316	85.9	1954	73.5			
middle school or comprehensive school	39	10.6	505	19.0			
upper secondary school (ref.category)	13	3.5	198	7.5			
Mother's vocational education					ns		
no vocational training	181	51.1	927	35.5			
vocational courses	79	22.3	577	22.1			
vocational school	63	17.8	586	22.5			
college level degree	24	6.8	394	15.1			
academic degree (ref.category)	7	2.0	126	4.8			
Participant's basic education					ns		
elementary school	42	11.0	137	5.1			
middle school or comprehensive school	252	66.0	1740	64.5			
upper secondary school (ref.category)	88	23.0	821	30.4			
Childhood home							
having well water	259	68.2	1531	55.8	ns		
sharing bed with someone	112	29.5	489	17.9	ns		
no. of family members					0.040	1.08	1.004- 1.16
no. of persons in bedroom					0.038	1.13	1.01-1.26
Smoking							
current smoking**	144	37.7	738	26.8	0.022	1.38	1.05-1.83
Alcohol consumption							
current alcohol consumption**	292	76.8	1755	63.8	0.032	1.40	1.03-1.91
Anfavourable situation¹ of life related to...							
economical situation ¹	360	95.5	2380	87.7	ns		
housing conditions ¹	270	71.2	1627	59.6	0.049	1.35	1.002-1.81
overall situation ¹	307	81.0	1980	72.6	ns		
¹ very good or good / average, bad or very bad							
Occurence of GORD-symptoms**	189	55.9	1002	42.0	ns		
Medical examinations and sick leaves because of dyspeptic symptoms							
Gastroscopy	44	11.6	170	6.2	ns		
X-ray of ventricle	51	13.5	220	8.0	ns		
being on sick leave**	11	2.9	34	1.2	0.009	3.36	1.35-8.33
<i>H. pylori</i> associated diseases							
gastritis	28	7.3	76	2.8	0.009	2.08	1.20-3.60
peptic ulcer disease	16	4.2	22	0.8	ns		

*For binary logistic regression analyses, the variables measuring educational level were entered as categorical variables, the crowding variables were entered as continuous variables and other variables as dichotomical variables. SPSS 12.0 software packages (SPSS, Inc., Chicago, Ill.) was used.

** The question was related to the last six months.

Table 14. Daily no. of cigarettes among smokers, and weekly amount of alcohol consumption in the last six months in *H. pylori*-positive and -negative young adults. Information collected by a questionnaire at the beginning of the 'screen-and-treat' programme.

	<i>H. pylori</i> positive		<i>H. pylori</i> negative		P-value*
	n	%	n	%	
daily no. of cigarettes					0.001
0 - 14	89	61.0	598	74.6	
15 - 30	56	38.4	199	24.8	
> 30	1	0.7	5	0.6	
weekly units of alcohol					<0.0001
0	88	23.2	997	36.2	
1 - 2	115	30.3	858	31.2	
3 - 7	98	25.8	572	20.8	
8 - 20	62	16.3	252	9.2	
> 20	17	4.5	73	2.7	

* Mann-Whitney's U Test's Exact Sig. (2-sided)

Reflux-symptoms and other gastrointestinal symptoms

The occurrence of GORD symptoms was significantly associated with *H. pylori* infection. Not only the frequency but also the severity of GORD-symptoms were higher among *H. pylori*-positive participants (Table 15).

We could not find a significant difference between antibody-positive and antibody-negative subjects in the severity or frequency of the other evaluated gastrointestinal symptoms (stomach cramps, feeling of swelling in upper abdomen, regurgitation of gastric juice, feeling of hunger, nausea, belching, diarrhoea, constipation or repeated urgent need for defecation).

Table 15. Occurrence of GORD-symptoms in the last six months in *H. pylori*-positive and *H. pylori*-negative young adults. Information collected by a questionnaire at the beginning of the 'screen-and-treat' programme.

	<i>H pylori</i> positive		<i>H pylori</i> negative		P-value*
	n	%	n	%	
severity					<0.0001
no harm	149	44.1	1382	58.0	
mild	69	20.4	454	19.0	
moderate	91	26.9	446	18.7	
severe	26	7.7	92	3.9	
very severe	3	0.9	10	0.4	
frequency					<0.0001
never or < once a month	196	53.3	1789	66.6	
1-3 times / month	108	29.3	617	23.0	
once a week	33	9.0	134	5.0	
2-6 times / week	21	5.7	112	4.2	
every day	10	2.7	34	1.3	

* Mann-Whitney's U Test's Exact Sig. (2-sided)

Medical examinations and sick leaves

Self-reported medical examinations (gastroscopy and/or x-ray of ventricle) and sick leaves due to upper abdominal symptoms were associated with *H. pylori* infection (Table 13). There were no significant differences between *H. pylori*-positive and *H. pylori*-negative participants in the number of doctor consultations or medications because of upper abdominal symptoms, and in the use of ASA and NSAIDs.

H. pylori associated diseases

A history of peptic ulcer disease and gastritis were associated with *H. pylori* infection (Table 13). One (0.3%) *H. pylori*-positive participant and two (0.1%) *H. pylori*-negative participants reported history of gastric cancer ($p = 0.321$). Whether mother or father had suffered from peptic ulcer disease did not reach statistical significance, although 43 (15%) and 244 (10.5%) of the *H. pylori*-positive and -negative participants, respectively, reported father's peptic ulcer disease, and 21 (6.6%) and 93 (3.8%), respectively, that of mother's. There were no significant differences between

H. pylori-positive and -negative participants in the occurrence of upper abdominal pain before the age of 16.

Situation in life and quality of life

We monitored the situation in life by a 5-graded scale (very good, good, average, bad, and very bad) and found that a more unfavourable (average, bad or very bad) situation in life was associated with *H. pylori* infection when related to economical situation, housing conditions, and overall situation (Table 13), but not when related to general health or health concerning stomach, daily work, family life, human relations and free time. We evaluated the quality of life by a 5-graded scale and found no significant differences between *H. pylori*-positive and -negative subjects in enjoying daily activities, sleeping, optimism about future, ability to concentrate on daily work/studying, decision-making, and happiness.

Independent risk factors for *H. pylori* infection

In multivariate analysis, the mother's basic education, number of family members and number of persons in bedroom in childhood household, current alcohol consumption, current smoking, housing conditions, being on sick leave and self-reported gastritis remained statistically significant as independent risk factors for *H. pylori* infection (Table 13b).

DISCUSSION

Screening and treating for *H. pylori* programme (Study I)

The primary health care system in Finland provided excellent opportunities to organize a large-scale screening and treatment programme. Three quarters of the invited 6109 subjects participated in our studies (88% in the pilot study, 74% in 1996 and 76% in 1997-2000) and the proportion of participating females was only slightly

higher than that of men. The participation rates were clearly superior to those of some previous reports, although the infected subjects were required to pay most of the expenses of the medicines used in eradication treatment themselves. In Bristol, UK in a double-blind placebo-controlled trial, only 39% of the invited unselected 20–59 year-old individuals participated in screening (Lane J et al., 2002). In Leeds, UK the participation rate was even lower, at 26%, when randomly selected individuals aged 40–49 years were invited (Moayyedi et al., 2000a). In Odense, Denmark, the participation rate was 63% in a study where 20,000 individuals aged 40 to 65 years and representing general population were randomized to screening and eradication for *H. pylori* or to a control group (Wildner-Christiansen et al., 2003). The studies in Bristol, Leeds and Odense assessed the effect of *H. pylori* screening in the community. However, the studies in Leeds and Bristol mainly concentrated on the question whether to treat or not to treat *H. pylori*-positive individuals in general, whereas the Danish study, like our own study, focussed on the question whether to screen or not to screen on a population level (Hansen et al., 2008).

Of note is the high acceptance of antimicrobial therapy for the eradication of *H. pylori* infection. The eradication rates for first-line therapy were 89.8% in the pilot study, and 82.2% and 77.6% per-protocol and 71.9% and 62.9% in an intention-to-treat analysis in subjects invited in 1996 and 1997–2000, respectively. Resistance to metronidazole was later demonstrated to be common among Finnish *H. pylori* isolates (Koivisto et al., 2004) and thus, antimicrobial resistance was probably the major cause for treatment failures. A longer duration of therapy or higher PPI doses could possibly have increased the eradication rates, when taking into account host related PPI metabolism and a high bacterial burden with *H. pylori* (Graham and Shiotani, 2008). However, the triple-therapy used in our study was in line with the first Finnish guidelines for the management of *H. pylori* infection in 1996 (Färkkilä et al., 1996). On the basis of this active intervention, the initial *H. pylori* seroprevalence rates were calculated to have decreased from 39% to 19% in the pilot study, from 12% to 4% among subjects aged 15 to 40, and in the extension of the study, from 3% and 27% among the 15 and 45-year-old subjects to 2% and 12%, respectively.

We started the voluntary ‘screen-and-treat’ programme before the first European guidelines for the management of *H. pylori* infection (EHPSG, 1997) were published.

According to these guidelines, it was recommended to screen and treat young dyspeptic patients without alarming symptoms by primary care physicians for *H. pylori* infection using UBT or locally validated serology in screening, but screening of asymptomatic individuals was not recommended. According to the latest update of the European guidelines, the Maastricht III Consensus Report, *H. pylori* 'test-and-treat' is appropriate for patients with uninvestigated dyspepsia (Malfertheiner et al., 2007). In high prevalence populations this is the strategy of choice in adult patients suffering from functional dyspepsia, while in areas of low *H. pylori* prevalence (<20%) the strategy is less effective and empirical acid suppression treatment with PPIs is recommended as an equivalent option (Malfertheiner et al., 2007). In our study, where also asymptomatic individuals were included, the prevalence of *H. pylori*-infection increased by age-group. In the pilot study, most of the participants represented a high prevalence population, while most of the participants in the 'screen-and-treat' programme from 1996 on represented a low prevalence population, since the seroprevalence rate was less than 20% among 15-35 year-old subjects in both of the study-phases.

The prevalence of dyspepsia is about 15 to 50% (Kay and Jørgensen, 1994; Agréus et al., 1994; Ford et al., 2007), and between 33 and 56% of the patients suffering from dyspepsia are infected with *H. pylori* (Bernersen et al., 1992; Agréus et al., 1995; McColl et al., 1997). It has been calculated, that about 10 % of the patients suffering from functional dyspepsia could benefit from successful *H. pylori* eradication treatment (Moayyedi et al., 2005), and that 30 individuals with *H. pylori* would have to be treated to prevent one person consulting their doctor for dyspepsia after two year's follow-up (Lane et al., 2006). It seems probable that a much longer follow-up period after eradication treatment is needed to show a more significant reduction in total dyspepsia-related health care costs (Ford et al., 2005). Although the prevalence of *H. pylori* infection was low at the base-line and the fact that there were also asymptomatic persons in our study, individuals successfully treated obviously benefitted from the treatment, and moreover, also the risk of possible complications of the infection like peptic ulcer disease, atrophic gastritis and gastric cancer is reduced. It will be seen in future, how much this intervention has affected the incidence and health-costs of these complications. On the other hand, possible side-effects with eradication therapy and anxiety due to awareness of *H. pylori* infection may have

troubled the participants, although severe adverse effects with eradication therapy have been seldom reported (Lee et al., 2006).

Indications for *H. pylori* eradication have widened. According to the latest European guidelines (Malfertheiner et al., 2007), the recommended indications for *H. pylori* eradication are: peptic ulcer disease, MALT lymphoma, atrophic gastritis, gastric cancer resection, gastric cancer in a close relative, long term maintenance treatment with PPIs, functional dyspepsia, unexplained iron deficiency anaemia, idiopathic thrombocytopenic purpura, and the start of long term maintenance treatment with NSAIDs. It is well-known that about 95% of the duodenal ulcers are caused by *H. pylori* infection, and successful *H. pylori* eradication prevents the recurrence of the ulcer disease (Ford et al., 2004). Of gastric ulcers commonly associated with the use of NSAIDs, about 70% are caused by *H. pylori* (Ford et al., 2004). In Finland, the prevalence of *H. pylori* associated peptic ulcer disease has declined (Veijola et al., 2005a; Koivisto et al., 2005), due to declining prevalence rate of *H. pylori* infection and to active antimicrobial treatment for the infection. In our study, peptic ulcer disease was detected in 12.1% of the infected patients and in 1.2% *H. pylori*-negative patients ($p < 0.0001$) (Study II). In rarely occurring MALT lymphoma, 62% of the patients with a low grade gastric MALT lymphoma demonstrated a complete remission after successful eradication (Malfertheiner et al., 2007). In about one half of those infected with *H. pylori*, gastritis proceeds to atrophic gastritis resulting in a loss of mucosal glands and decreased helicobacter colonization, and to intestinal metaplasia, which are regarded as precursors for gastric cancer (Kuipers et al., 1995; Valle et al., 1996; Lauwers, 2003). The development of atrophic gastritis can be stopped by *H. pylori* eradication, and atrophic changes on gastric mucosa can turn to normal after successful eradication (Kokkola et al., 2002).

H. pylori infection has been classified as a first class carcinogen for gastric cancer (International Agency for Research on Cancer, 1994). It is estimated that 60-90% of gastric cancers are associated with *H. pylori* (Malfertheiner et al., 2005). In Finland, the decline in the local prevalences of *H. pylori* infection appeared to predict the declining local incidences of gastric cancer after a median latency of 20 years (Rehnberg-Laiho et al., 2001). In reports on the effect of *H. pylori* eradication on the development of gastric cancer, the development of cancer was prevented only in

patients without atrophic gastritis and intestinal metaplasia before eradication therapy (Wong et al., 2004). Although it has been suggested that *H. pylori* eradication for gastric cancer prevention should be evaluated in populations at risk (Malfertheiner et al., 2007), screening and treating the general population for *H. pylori* has not yet been recommended. By adopting the ‘screen-and-treat’ programme for young adults, as presented here, it may be possible to cure the infection before the development of pre-neoplastic conditions and also the transmission of the infection from parents to children may be prevented.

An epidemiological questionnaire was returned by 99.7% of the participants in 1996. Our results on the risk factors of *H. pylori* infection were in agreement with reports from elsewhere (Malaty and Graham, 1994; Everhart et al., 2000; Koch et al., 2005; Bures et al., 2006) and the results from the multivariate analysis confirmed that a low education of the mother and crowded living conditions in the childhood household were also in Finland associated with *H. pylori* infection. In addition, multivariate analysis showed that current smoking, current alcohol consumption, housing conditions, and being on sick leave due to dyspeptic symptoms remained statistically significant as independent risk factors for *H. pylori* infection in our study. *H. pylori* infection seemed to be a good indicator for an individual’s socioeconomic situation, as the economical situation, housing conditions and the overall situation of life were found in univariate analyses to be significantly more favourable in *H. pylori* negative subjects. Although most of the significant risk factors in univariate analysis were independently associated with *H. pylori* infection in multivariate analysis, many of them could possibly reflect a lower socioeconomic status in childhood household.

Special role of serology (Studies I-IV)

Our research group has used serology for many years and it has proved to be a highly accurate method both in diagnosing *H. pylori* infection (Kosunen et al., 1992, Oksanen et al., 1998) and in confirming the treatment success (Kosunen et al., 1992, Veijola et al., 2005a). On the basis of our extensive experience in serology this method was also chosen for screening in the present study. Recommendations for the diagnostic methods used for detecting *H. pylori* infection have changed and now it is

generally accepted that the role of serology in detecting the infection is crucial. The latest European guidelines on the management of *H. pylori* infection, the Maastricht III Consensus Report underlined the special role of serology compared to other diagnostic tests (Malfertheiner et al., 2007), since PPI treatment prior testing can lead to false negative results in other diagnostic tests than serologic assays. Serological tests are recommended to assess *H. pylori* infection also in patients suffering from bleeding ulcers and in conditions with a low bacterial density (gastric atrophy, MALT lymphoma); both invasive and other non-invasive tests have demonstrated a limited sensitivity in those patients. However, office-based serological tests or near patient tests are not recommended due to their low accuracy (Malfertheiner et al., 2007).

Although the best quantitative serological tests show high sensitivity and specificity, all tests are not equal in accuracy and it is recommended to validate antibody assays locally (Feldman et al., 1995; Herbrink and van Doorn, 2000). The accuracy of a diagnostic test is strongly dependent on the prevalence of *H. pylori* infection (Leodolter et al., 2001; Oksanen et al., 2001). In areas of low *H. pylori* prevalence, the diagnostic test used may demonstrate a lower positive predictive value resulting in an increased number of false positive test-results. We evaluated the EIAs used in our studies (I, II, III, IV) in different age-groups (Study II). Both the in-house and commercial EIAs demonstrated high specificity in subjects below 50 years of age, with a low prevalence rate of *H. pylori* infection. However, serologic tests may have a lower accuracy if the population has been actively treated for *H. pylori* infection; after successful *H. pylori* eradication therapy, the antibodies decline slowly to the normal level staying elevated even for ten years in some of the successfully treated patients (Veijola et al., 2007). In our study (Study I), in the case of earlier treated participants, UBT was used to verify the serologic results to confirm the diagnosis of *H. pylori* infection.

Follow-up after treatment is recommended and the latest European guidelines (Malfertheiner et al., 2007) advise to confirm the eradication treatment for at least four weeks after treatment using UBT. If UBT is not available, according to the recommendation, a laboratory-based stool test using monoclonal antibodies could be used in assessing the success of treatment. Locally validated serologic assays with a high specificity and sensitivity as used in our study (Study I) provide a reliable, easy

and cheap method, not only to diagnose *H.pylori* infection (Kosunen et al., 1992; Herbrink and van Doorn, 2000), but also to confirm the success of treatment (Kosunen et al., 1992; Rautelin and Kosunen, 2004). Using serology as a follow-up method after treatment requires taking a pre-treatment serum, a post-treatment serum four to six months after eradication therapy and testing the samples in parallel in the same assay to determine the decline of antibody titres. In our study (Study I), we confirmed in the population-based 'screen-and-treat' study the treatment failures by using UBT in most cases at least before the possible second course of treatment and found only one individual with a discrepancy between serology and UBT.

Age-dependent accuracy of *H. pylori* antibody assays, with special reference to atrophic gastritis (Study II)

Patients with atrophic corpus gastritis often have positive *H. pylori* serology, although microscopical examination (Karnes et al., 1991; Testoni et al., 1996), culture of biopsy samples, and even the UBT remain *H. pylori* negative (Kokkola et al., 2000). These particular patients may still be infected as demonstrated by rapidly falling antibody titres after therapy (Kokkola et al., 1998). We evaluated (Study II) the accuracy of the EIAs used in our studies (I-IV) regarding to the presence of atrophic gastritis. Atrophic gastritis was detected in 37% of the infected patients and in 14% of the *H. pylori*-negative patients and it was more common in older age-groups appearing in 10%, 17% and 40% of the patients aged 15 to 49, 50 to 64, and >64 years, respectively. The sensitivities of the both IgG tests were high in all age-groups, but the specificities for in-house and Pyloriset IgG tests declined by age. However, the lower specificities for the older age-groups might be due to false negative results of the reference tests (histology and culture), in which mucosal atrophy with a diminished bacterial density can lead to false negative test results (Malfertheiner et al., 2007). The exclusion of 'false' positive results for patients with atrophic gastritis improved the specificities for both of the tests in older age-groups. In our study, *H. pylori* positivity was defined as a positive result in histology or culture. These invasive methods seemed to show a lower sensitivity in detecting *H. pylori* infection in patients with atrophic gastritis, supporting the special role of serology in

diagnosing *H. pylori* infection in patients with atrophic gastritis, according to the latest European Guidelines (Malfertheiner et al., 2007).

Changes in IgA and CagA levels (Studies III and IV)

H. pylori infection induces an immunologic response with circulating antibodies which is not able to eradicate the bacterium from the gastric mucosa. Most infected subjects have specific circulating IgG antibodies, but systemic IgA antibodies for *H. pylori* are elevated in approximately two-thirds of the infected subjects (Kosunen et al., 1992), and 2-7% of the infected individuals show an elevated IgA level alone (Kosunen et al., 1992; Kosunen et al., 1997; Jaskowski et al., 1997). The prevalence rates of *H. pylori* antibodies increase by age (Kosunen et al., 1989; Andersen et al., 1996), due to the so called cohort phenomenon of *H. pylori* infection (Banatvala et al., 1993a; Kosunen et al., 1997). The sensitivity of *H. pylori* IgA assays is reported to be lower than that for IgG, especially in younger age-groups (Kolho et al., 2002). Also in our studies, the seroprevalence rate of *H. pylori* infection, as measured by *H. pylori* IgG and IgA antibody results combined, increased by age (Study I) in 1994 from 5 % to 69% in 15 to 75-year-old subjects, and in 1996 from 4 % to 22% in 15 to 40-year-old individuals in the 5-year age groups. The sensitivities of the both IgA tests were low among those aged 15 to 49 years, but clearly increased by age, for the commercial EIA from 52% among those aged 15 to 49 to 86% among those aged 65 or more.

Serological tests, especially those detecting antibodies against the specific immunogenic antigen CagA, are regarded as the best method to document the link of *H. pylori* infection with gastric cancer (Cover et al., 1995; Malfertheiner et al., 2007). *H. pylori* strains are either CagA-positive or CagA-negative based on whether they possess the CagA protein encoded by the *cagA* gene. CagA is present in 40 to 70% of *H. pylori* strains, and it is associated with peptic ulcers, precancerous conditions and gastric cancer in the West according to several studies (Cover et al., 1995; Kuipers et al., 1995; Parsonnet et al., 1997; Kusters et al., 2006). Furthermore, an IgA antibody response during *H. pylori* infection has also been associated with peptic ulcer disease

and gastric cancer (Aromaa et al., 1996; Kosunen et al., 2005), and production of IgA antibodies has been associated with a CagA-positive infection (Rautelin et al., 2000).

We studied the changes in the prevalence rates and levels of *H. pylori* IgA antibodies (Study III) and CagA antibodies (Study IV) in two decades' follow-up by utilizing frozen serum samples. The use of frozen sera was based on the fact that *H. pylori* antibodies tolerate multiple freezings and thawings (Pearce et al., 1996, Blaser et al., 1991).

We showed (Study III) that the increase in the prevalence rate of *H. pylori* IgA antibodies according to age (Kosunen et al., 1989; Andersen et al., 1996) was due not only to the birth cohort phenomenon and seroconverters but also to rising IgA titres during chronic infection. In several subjects, the initial normal IgA antibody levels later rose above the cut off level, increasing the proportion of IgA seropositive subjects in older cohorts. The maturation of the IgA response in *H. pylori* infection appeared to continue into adulthood. Interestingly, IgA production, seen both in a greater number of responders and as higher IgA titres has been found to be stronger in children infected with a CagA-positive strain than in children with CagA-negative strains (Kolho et al., 1999).

The seroprevalence rate of CagA antibodies (IV) declined significantly during the two decades preceding follow-up, which paralleled the overall decline in *H. pylori* seroprevalence in Vammala during that time (Kosunen et al., 1997). Furthermore, the decline in seroprevalence of CagA-positive *H. pylori* strains in subjects aged 14 to 44 years was significantly greater than that for *H. pylori* CagA-negative strains. This might be associated with the repeated antibiotic usage over the course of childhood (Blaser, 1999) and the evidence that CagA-positive strains were more susceptible than CagA-negative strains to eradication treatment (van Doorn et al., 1997). It remains to be seen in the coming years whether the more rapid decline of *H. pylori* CagA-positive strains will affect the prevalence rate and clinical importance of IgA antibodies, as well as the outcome of severe complications of *H. pylori* infection.

Changes in overall *H. pylori*-positivity and CagA antibody status were also studied by utilizing the paired sera from 221 subjects obtained both in 1973 and 1994 (Study IV).

In total, only 5.4% of subjects had a change in overall *H. pylori* status during the two decades follow-up period giving a crude seroconversion rate of 0.4% and a crude seroreversion rate of 0.13% per year. In addition, nearly identical serum antibody levels to CagA and to the acid glycine extracted antigen (in-house IgG and IgA) in the same individuals 21 years apart were found, which confirms the stability of antibody responses and the longstanding equilibrium between hosts and their *H. pylori* populations (Blaser and Kirschner, 1999).

The long-term changes in the levels of *H. pylori* IgA antibodies were measured both in Study III and Study IV utilizing in part the same frozen serum samples. However, the study designs of these two studies were different in three aspects; 1) *H. pylori* positivity was defined in different way. In Study III, the persisting *H. pylori* infection was defined as a positive IgG antibody result at the beginning and at the end of the follow-up period. When evaluating the changes in antibody levels and antibody status, the positivity was based on antibody level exceeding the cut-off value for the test evaluated. In study IV, *H. pylori* positivity was defined as a positive result in any of the three serological assays used (in-house IgG, in-house IgA and CagA). *H. pylori*-negative status was defined when all three assays were negative. 2) The changes in the antibody levels were calculated as median titres/values in Study III and as mean titres in Study IV. 3) When evaluating long-term changes in titres, the criteria for included subjects were different. In Study III, only those subjects with a persisting positive IgG and IgA response were included, while in Study IV, all subjects who were IgG-seropositive in the beginning of the follow-up were included.

Public health perspective (Study I)

H. pylori infection is related to gastric cancer, associated with high morbidity and poor prognosis, and peptic ulcer disease, which demonstrates high morbidity and modest mortality. The information on *H. pylori* infection has not been effective at primary care level, according to some reports; In a worldwide survey, of the GPs, 81%, 85% and 61% knew the causal relationship of *H. pylori* with gastric and duodenal ulcers and gastric cancer, respectively (Huang et al., 2003). In a study from England, only 10% of those patients who had peptic ulcer disease had been prescribed

eradication therapy (Wright et al., 2001). There are large numbers of people who have suffered from a documented peptic ulcer but who have not been treated for *H. pylori*. In young people the prevalence of *H. pylori* is low and still declining and the prevalence of peptic ulcer disease is low. Since ulcer disease has a high prevalence in older people, it has been suggested, that if we aim to eliminate ulcer disease, we have to focus on the prevalent cases (Lai et al., 1999, de Boer 2000) or target the population who regularly uses acid suppressants (deBoer 2000).

H. pylori eradication seems to be the most practical means of preventing gastric cancer and peptic ulcer disease, but population screening among asymptomatic individuals is suggested only for populations with a high risk for gastric cancer and among those individuals also listed in the latest European guidelines appropriate for eradication therapy (Forman and Graham, 2004, Malfeltheiner et al, 2007). One approach for screening and treatment for *H. pylori* infection in populations at risk could be to focus on migrant populations in the West, among which the prevalence of the infection is high (de Vries et al., 2008).

Intervening in otherwise healthy individuals requires critical analysis to weigh up the possible harms of a ‘screen-and-treat’ intervention. From a public health perspective, induction of antimicrobial resistance due to antibiotic combinations used in eradication is expected to be of less importance than that of antibiotic prescription for other reasons, since metronidazole, amoxicillin and clarithromycin as sole treatments are more commonly prescribed for other indications in a society. According to the hypothesis that *H. pylori* could be one of the microbes counteracting atopy, an increase in IgE-based allergy may occur in a population actively treated for *H. pylori* infection (Kosunen et al., 2002).

Although the prevalence rate of *H. pylori* infection has decreased in Finland (Kosunen et al, 1997), and the prevalence of *H. pylori* associated peptic ulcer disease has declined in Finland (Veijola et al., 2005a; Koivisto et al., 2005), the severe consequences of the infection still exist. Indications for eradication treatment have widened and also public awareness of the infection and associated complications has increased the need for testing and treating it. In Finland in ordinary clinical practise, all patients found to be infected with *H. pylori* can receive an eradication treatment

when requested. From a public health perspective *H. pylori* screening and treating has the potential to reduce the burden of peptic ulcer disease and possibly that of dyspepsia in the community and will presumably prevent gastric cancer, if the target population is young adults as in our study. Although the prevalence of *H. pylori* infection was low even before the intervention, it remains to be seen in the coming years, how much this intervention will affect the prevalence and related health costs of *H. pylori* associated diseases.

CONCLUSIONS

1. The decline of *H. pylori* gastritis was accelerated by the programme. The primary health care system of Finland provided an excellent opportunity to organize this large-scale 'screen-and-treat' programme. In the pilot study, the initial seroprevalence rate of 39% was calculated to have declined to 19% after the intervention. In the 'screen-and-treat' programme, the initial seroprevalence rate of 12% among subjects aged 15 to 40 years in 1996 was calculated to have decreased to 4%, and the initial seroprevalence rates of 3% and 27% among subjects aged 15 and 45, respectively, in 1997-2000 were calculated to have decreased to 2% and 12% after the programme.
2. Crowding variables in the childhood household, low education of the mother, smoking habits and alcohol consumption, unfavourable housing conditions, and sick leave due to dyspepsia were statistically significant independent risk factors for *H. pylori* infection in this young Finnish adult population.
3. The IgG antibody test was highly accurate in subjects aged 15 to 49, but the specificity declined in older age-groups, in which the prevalence of atrophic gastritis increased. The sensitivity of the IgA test increased and the specificity decreased by age. The exclusion of 'false' positive results for patients with atrophic gastritis clearly improved the specificities of both the tests for older age-groups.
4. The increase in the number of IgA-antibody-positive *H. pylori* patients with age was due not only to the birth cohort phenomenon and seroconverters but also to rising IgA titres during chronic infection. In several initially IgG-antibody-positive subjects, the initial normal IgA antibody levels later rose above the cut off-level, increasing the proportion of IgA seropositive subjects in older cohorts. The elevated IgA antibody levels significantly rose during infection.
5. The proportion of subjects infected with CagA⁺ *H. pylori* strains as measured by CagA antibodies had declined more rapidly than the proportion of subjects infected with CagA⁻ *H. pylori* strains in subjects aged 14 to 44, as the overall prevalence of *H. pylori* has declined.

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